

CHEST COMPUTED TOMOGRAPHY IN EARLY AND ADVANCED CYSTIC FIBROSIS LUNG DISEASE

Optimizing protocols, image analysis and further validation

Martine Loeve

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Chest Computed Tomography in early and advanced cystic fibrosis lung disease

Optimizing protocols, image analysis and further validation

Thorax Computer Tomografie bij milde en vergevorderde long ziekte door cystische fibrose

Optimalisatie van protocollen, beeld analyse en verdere validatie

Proefschrift

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Never too far

*One can be close
Together
And still not see
The deepest of heart
Of a partner to be*

*One can be far
Apart
But feel despite
True connection
With the love insight*

*A love as strong to
Overcome
All distances means
A heart
Never too far*

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Chapter 1

General Introduction

Cystic Fibrosis

Cystic Fibrosis (CF) is one of the most common life-threatening autosomal recessive diseases in the Western World, with a reported incidence rate of approximately 1 in every 2,000 to 5,000 caucasian newborns in most European countries. (1-3) The gene mutation that plays an important role in the pathophysiology of CF is a mutation in the cystic fibrosis transmembrane conductor regulator (CFTR) gene on chromosome 7. Currently, 1,890 mutations in this gene are known to be associated with CF, from which the F508 deletion is the most prevalent mutation. (4) The CFTR gene codes for the CFTR protein which is present in the membrane of epithelial cells, and functions as an important regulator of the surface fluid in the airways. (5-6) Mutations in this gene result in defective chloride secretion and excessive sodium reabsorption, which negatively impacts the airway surface fluid and impairs the mucociliary clearance. (7) This may form the basis for the chronic bacterial airway infection and inflammation characteristic for CF, which results in irreversible lung damage. (5) However, the pathophysiology of CF is not completely understood and the mechanism of acquisition and maintenance of bacterial infection in the CF airway is unclear. In addition, there is a poor correlation between genotype and phenotype, especially with the severity of lung disease. (8) Thus, knowing which mutation causes CF is of little help in predicting the course of the disease.

Lung disease in CF

Although CF affects multiple organs, it is the severity of lung disease that causes most of the disease-related morbidity and mortality. At birth, the lungs of CF patients appear to be histologically normal. (8) However, CF lung disease starts early. Cohort studies have shown the presence of structural abnormalities in the lungs of infants with CF, even in the absence of symptoms. (9-12) Frequently occurring

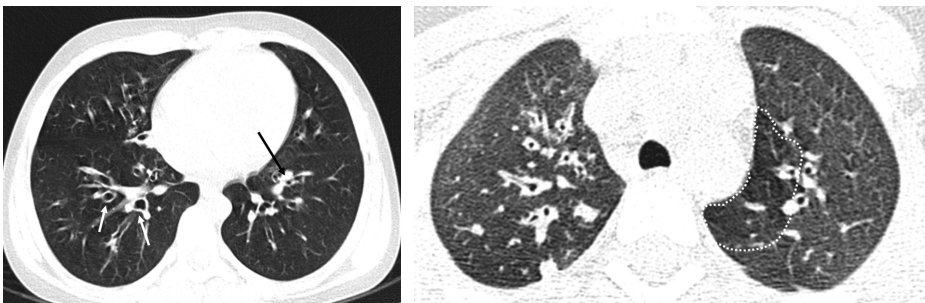


Figure 1. Computed tomography images of an 8 year-old boy showing the frequently occurring structural abnormalities in the lungs of CF patients. Shown are bronchiectasis and airway wall thickening (A, white arrows), mucus plugging (A, black arrow), and trapped air (B, dashed area)

lung abnormalities in CF are bronchiectasis, airway wall thickening, mucus plugging, trapped air (TA), atelectasis/consolidations, and bulla/cysts (Figure 1).

Monitoring CF lung disease

To diagnose and monitor the progression of structural lung changes and their response to treatment, several diagnostic modalities are being used in clinical practice. These modalities can be categorized into pulmonary function tests (PFTs) to measure functional aspects of the lung and imaging techniques to visualize lung structure.

Pulmonary function tests

For CF, the most routinely used PFTs are spirometry and body plethysmography. From spirometry, the forced expiratory volume in 1 second (FEV_1) is considered an important surrogate outcome measure of CF lung disease. PFTs such as spirometry require optimal patient cooperation, and can therefore only be performed in cooperative children aged 6 years and older. Alternative PFTs methods have been developed to measure lung function in infants and preschool children.(13-14) For young children, the most widely used method is the body box. Unfortunately, this method is cumbersome, variable, and time-consuming and therefore mostly used in a research setting. More recently the lung clearance index (LCI), derived from multiple breath washout techniques, has been used. The LCI is reported to be sensitive to detect small airways disease (15), which is thought to be an early marker of CF lung disease.(16) However, further validation to determine the clinical relevance of LCI as a diagnostic and monitoring tool for CF-lung disease is needed. (17)

Chest imaging

To determine lung structure, various imaging modalities can be used. Traditionally, chest radiography was considered the most important imaging modality to diagnose and monitor CF-related structural abnormalities. However, chest radiography has a low sensitivity to detect early changes and to monitor disease progression.(18-19) Currently, chest computed tomography (CT) is the most sensitive imaging modality to detect structural changes in the lungs. More recently, chest magnetic resonance imaging (MRI) has been suggested as a radiation free alternative for chest CT. The sensitivity of MRI to depict large morphological changes has been estimated to be comparable to CT.(20) However, its sensitivity to detect early and smaller changes in lung structure is considered to be inferior to that of chest CT.(20-22) In this thesis, we focused on CT protocols, image analysis of chest CT scans and validation of CT derived parameters as outcome measures for CF lung disease.

CT Protocol

CT is currently the modality of choice when it comes to assessment of structural changes in CF. CT has the advantage of being more sensitive for detection and monitoring of CF-related structural abnormalities compared to PFTs. (19-20) Furthermore, CT scanning can be performed in infants, using either free breathing or controlled ventilation techniques.(23) The disadvantage of CT is that it exposes patients to ionizing radiation, which increases a patient's life long risk of cancer. Therefore, CT protocols should aim to limit radiation dose to the absolute minimum needed to acquire images of sufficient quality. Unfortunately, relatively little research has been performed on how to acquire all relevant information at the lowest radiation dose. Most routine CT protocols for CF include images acquired after maximal inhalation (inspiratory images) and images acquired after maximal exhalation (expiratory images). Inspiratory images are used to diagnose structural changes such as bronchiectasis. Expiratory images are needed to evaluate TA. However, it is unknown whether expiratory scans may suffice for the assessment of structural changes as well. When expiratory images would suffice to obtain all clinically relevant information, radiation dose would be substantially reduced. In addition, the optimal expiratory protocol for detection of TA is unknown. It is common practice to use only 3 expiratory slices to assess TA.(9, 11-12, 24-29) However, whether this approach is sensitive enough for accurate assessment of TA is not clear, as the effect of the number of slices on TA assessment has never been systematically studied in CF.

Image analysis

Further validation of CT requires quantification of the structural abnormalities on CT. A method used to date is semi-quantitative scoring, for which several reproducible methods have been developed.(30) However, a major disadvantage of these systems is that it is difficult to understand the clinical relevance of the scores. Scores expressed in percentage of total lung volume would be easier to understand. In addition, these systems were mainly developed for mild to moderate CF lung disease. A specific scoring system to quantify abnormalities in severe advanced lung disease (SALD) is not available. In addition, little is known about the spectrum of structural abnormalities in CF patients with SALD. A dedicated SALD scoring system is probably more sensitive to detect differences in the disease spectrum between these patients, and could be used to study the structural abnormalities in SALD.

Validation

Due to its extensive validation, FEV₁ has long been considered the most clinically relevant surrogate outcome parameter to detect and monitor CF lung disease in clinical practice and clinical trials. However, due to advances in treatment, life expectancy has greatly increased over the years. Currently, the median age of CF patients is approaching 38 years.(31) This improved survival is paralleled with a reduced annual loss in FEV₁. Hence, FEV₁ has become a relative insensitive parameter to monitor disease progression and is therefore less suitable as a primary endpoint in clinical trials. Thus, there is a need for validated, sensitive and accurate outcome parameters for CF lung disease. CT has great potential to fulfill these requirements. First, chest CT is the gold standard to detect bronchiectasis, the most prominent structural change in CF lung disease. Second, CT has been shown to be more sensitive than PFT parameters to detect and monitor structural abnormalities such as bronchiectasis and airway wall thickening.(32) Third, various CT scores have been shown to respond to treatment. (27, 33-38) Fourth, recently CT parameters have been found to correlate with the true outcome measure health-related quality of life. (39-40) However, various important steps in the validation process were still missing. In the validation process, it is important to know the course of CT abnormalities over time. Bronchiectasis for example, has been shown to be irreversible and progressive in CF.(41) In addition, progression of airway wall thickening has also been reported.(32) For TA however, little is known about the course and reversibility over time. In addition, we aimed to study the correlation between chest CT and important clinical outcomes parameters such as respiratory tract exacerbation rate (RTE-R) and survival. The association between CT scores and RTE-R was only investigated in one small, selected cohort.(24) Whether CT-related parameters correlate with RTE-R in an unselected CF population was unknown. In addition, the link between CT and survival has never been investigated. Several studies have aimed to find better predictors of survival, but CT parameters were never evaluated.(42-47) If there is a link between CT scores and survival, the next step would be to investigate whether CT-related parameters can improve currently used prediction models for survival in CF.

Aim of the study

We executed 5 studies in patients with early and advanced CF lung disease with the aim to optimize CT protocols and image analysis, and to further validate CT parameters as surrogate endpoints in clinical trials.

Optimizing CT protocols:

- To investigate whether ultra low dose expiratory CT scans could suffice for assessment of CF-related structural lung abnormalities
- To investigate the effect of the number of CT slices on TA assessment in CF

Image analysis:

- To design a new quantitative CT scoring system for evaluation of CT scans of CF patients with SALD
- To investigate the spectrum of structural abnormalities in SALD patients using the SALD CT scoring system

Validation:

- To study the course and reversibility of TA over time in children with CF
- To investigating the predictive value of CT scores for RTE-R in children with CF
- To study the correlation between SALD CT scores and survival in CF patients with SALD awaiting lung transplantation
- To investigate whether SALD CT scores can improve currently used prediction models of survival for CF patients awaiting lung transplantation
- To review what is known and what is still missing in the validation process of CT as surrogate endpoint in CF clinical trials.

Outline of this thesis

Chapter 1 contains the introduction to the studies that were performed in this thesis.

Chapter 2 describes the results of a study in which low dose inspiratory and ultra low dose expiratory CT scores were compared to determine whether expiratory CT alone may suffice for monitoring the structural changes in CF lung disease.

Chapter 3 reports the results of a study investigating the effect of the number of expiratory CT slices on the assessment of TA in CF.

Chapter 4 provides the results of a study aiming to design a new quantitative CT scoring system for CF patients with SALD, and to determine the spectrum of structural abnormalities in SALD.

Chapter 5 reports the results of a study investigating localized changes in TA distribution over time using automated image analysis software.

Chapter 6 shows the results of a study investigating the association between CT scores and RTE-R in an unselected cohort of pediatric CF patients.

Chapter 7 shows the results of a study investigating the association between CT scores and survival, and the added value of CT to a currently used survival prediction model are shown.

Chapter 8 provides a review on what is known about the use of CT as surrogate endpoint in CF clinical trials and what further research is needed to complete the portfolio of CT.

Chapter 9 provides a general discussion on the results of the studies performed in this thesis.

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Part 1

Optimizing CT protocols in CF

Chapter 2

Cystic Fibrosis: Are volumetric ultra-low-dose expiratory CT scans sufficient for monitoring related lung disease?

Loeve M, Lequin MH, de Bruijne M, Hartmann IJC, Gerbrands K, van Straten M, Hop WCJ, Tiddens HAWM.

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Abstract

Purpose To assess whether chest computed tomography (CT) scores from ultra-low-dose end-expiratory scans alone could suffice for assessment of all cystic fibrosis (CF)-related structural lung abnormalities.

Methods In this institutional review board–approved study, 20 patients with CF aged 6–20 years (eight males, 12 females) underwent low-dose end-inspiratory CT and ultra-low-dose end-expiratory CT. Informed consent was obtained. Scans were randomized and scored by using the Brody-II CT scoring system to assess bronchiectasis, airway wall thickening, mucus plugging, and opacities. Scoring was performed by two observers who were blinded to patient identity and clinical information. Mean scores were used for all analyses. Statistical analysis included assessment of intra- and interobserver variability, calculation of intraclass correlation coefficients (ICCs), and Bland-Altman plots.

Results Median age was 12.6 years (range, 6.3–20.3 years), median forced expiratory volume in 1 second was 100% (range, 46%–127%) of the predicted value, and median forced vital capacity was 99% (range, 61%–123%) of the predicted value. Very good agreement was observed between end-inspiratory and end-expiratory CT scores for Brody-II total score (ICC=0.96), bronchiectasis (ICC=0.98), airway wall thickening (ICC=0.94), mucus plugging (ICC=0.96), and opacities (ICC=0.90). Intra- and interobserver agreement were good to very good (ICC range, 0.70–0.98). Bland-Altman plots showed that differences in scores were independent of score magnitude.

Conclusions In this pilot study, CT scores from end-expiratory and end-inspiratory CT match closely, suggesting that ultralow-dose end-expiratory CT alone may be sufficient for monitoring CF-related lung disease. This would help reduce radiation dose for a single investigation by up to 75%.

Introduction

In patients with cystic fibrosis (CF), lung disease is the predominant cause of morbidity and mortality (1). To enable early intervention and treatment, it is important to monitor the onset and progression of lung disease at an early stage (2) Pulmonary function tests (PFTs), combined with chest radiographs, were long considered to be the standard of care for monitoring CF lung disease (3). Recently, computed tomography (CT) was shown to be more sensitive for detection of disease progression than were PFTs (4-7). Furthermore, CT is superior to chest radiographs for depiction of structural abnormalities such as bronchiectasis (8-9). Most routine CT protocols for CF include inspiratory and expiratory images made during voluntary breath holds. End-inspiratory CT scans are used to monitor structural changes such as bronchiectasis, peribronchial thickening and consolidations. End-expiratory scans are used to evaluate trapped air (4, 10), which is an early and important feature in CF lung disease (7, 11-12).

The major disadvantage of the use of recurrent CT scanning to monitor CF lung disease is the repeated exposure of the patient to ionizing radiation. Since life expectancy for patients with CF is progressively increasing (13), lifelong exposure to ionizing radiation should be limited to the lowest possible dose (14). Current strategies for minimizing radiation from CT include low-dose protocols, automated patient centering in the gantry (15), and reduction of the number of images per scan (16-18). These strategies were developed primarily for use with end-inspiratory CT. To our knowledge, only one study has focused on dose reduction for end-expiratory CT (19). Recently, ultra low dose CT protocols were developed, which help to further reduce radiation dose.

At our center, biannual volumetric low-dose end-inspiratory CT and ultra-low-dose end-expiratory CT have been used since 2006. After assessing these scans for a year, it occurred to us that end-expiratory CT images might reveal the same structural abnormalities as observed on end-inspiratory CT. This led us to speculate that end-expiratory CT alone could suffice for detection of relevant CF-related changes. To our knowledge, no researchers have investigated the use of end-expiratory CT for evaluating structural changes other than trapped air. Furthermore, whether CT scores from end-inspiratory CT images are comparable to those obtained with end-expiratory CT images has not been studied. If end-expiratory CT is sufficient for monitoring CF lung disease, radiation dose could be substantially reduced, improving the risk-benefit ratio of the use of CT in patients with CF. Therefore, the aim of this pilot study was to assess whether chest CT scores from ultra-low-dose end-expiratory CT alone could suffice for assessment of all CF-related structural lung abnormalities.

Methods

Study population

Twenty consecutive subjects with CF who were monitored at a single tertiary CF clinic (Erasmus Medical Center Sophia Children's Hospital) were selected for this study. Patients were enrolled if they underwent routine biannual CT scanning and PFTs as part of their annual visit between March 2004 and August 2007. In addition, all patients had to be clinically stable. Children receiving intravenous antibiotics for pulmonary exacerbations were considered unstable and thus, were not included. The institutional review board approved the retrospective study protocol, and informed consent for retrospective use of anonymous data was obtained for all patients.

CT scanning procedures

Scans were performed with a 6-section CT scanner (Somatom Emotion; Siemens Medical Solutions, Erlangen, Germany) with the patients in supine position. Each CT examination consisted of one volumetric end-inspiratory CT and end-expiratory acquisition. All children received similar instructions for voluntary breath holds before scanning. For younger children however, more time was scheduled to explain the procedures to the child and to practice the breath hold instructions. A 0.6-second rotation time was used with a tube voltage of 80 kV (patients <35 kg) or 110 kV (patients >35 kg). Scanning was performed from lung apex to base, including the costophrenic sulci, with a 1.5 pitch and 6x2 mm collimation. Images were reconstructed with a 2.5 mm section thickness, 1.2-mm increment and a B60s kernel. For optimal image quality with the inspiratory protocol, traditionally used for assessment of CF-related structural changes, a modulating tube current (CareDose4D; Siemens Medical Solutions) with an effective (i.e. divided by pitch) reference tube current-time product of 20 mAs was used. For end-expiratory CT, used for trapped air assessment, image quality was considered sufficient when the tube current was fixed at 25 mA, with an effective tube current-time product of 10 mAs. As a result, the radiation dose for end-expiratory CT was lower than for end-inspiratory CT.

Lung volume measurements

CT volume levels for end-inspiratory CT and end-expiratory images were assessed by using a morphometric approach (M.L. 3 years of experience) with a precessing application (20). First, a 10x10-mm grid was digitally projected over each image. Second, the lung area in each section was estimated by counting the grid cells projected over lung tissue. The percentage filled was recorded for grid cells that were partially filled with lung tissue. Percentages were added, and the sum was rounded to the nearest whole number. This number was added to the number of completely filled cells. Third, the lung area on each section was multiplied by the increment to calculate the volume. Right and left lung volumes were added to compute total

lung volume. Scan volumes were then compared with body box measurements of lung volume. Total lung capacity (TLC), residual volume (RV), and functional residual capacity (FRC) were obtained by using a body plethysmograph (Masterlab; Erich Jaeger, Würzburg, Germany). Inspiratory scan volume was compared with TLC, and expiratory scan volume was compared with RV and FRC. All scan volumes were expressed as percentages of body box volumes, as these volumes were considered to be the maximum a child was able to achieve. Spirometry was performed by using a diagnostic system (Erich Jaeger). All reference values were obtained according to Zapletal et al(21).

CT evaluation

Scans were scored according to pattern and severity for bronchiectasis, airway wall thickening, mucus plugging, opacities, and trapped air by using the Brody-II CT scoring system (7). All components except trapped air were scored on both inspiratory and expiratory images. Trapped air was excluded because it can only be assessed on end-expiratory CT images. Therefore, the maximal possible total score (207 points) was reduced by the trapped air score (27 points), which changed the upper limit to 180 points. Scores were expressed as percentages of maximal possible scores on a scale of zero (no disease) to 100 (maximal lung disease). All scans were collected and scored in one batch. Identifying information was removed. End-inspiratory and end-expiratory CT scans were randomized and independently presented to two observers (K.G. and M.L., with 1 and 3 years experience, respectively, scoring CT scans from CF patients for study purposes) for scoring. Observers were blinded to clinical data. Intraobserver variability was established by having observer 2 rescore all CT scans after 1 month. Interobserver variability was established by using all CT scans; mean scores were used for analysis.

To quantify the differences in radiation dose between end-inspiratory and end-expiratory CT, the dose-length product for both scans was obtained from the CT scanner console for each patient (22). In addition, mean effective dose (expressed in milliSieverts) for both scans was calculated by using a dosimetry calculator (ImPACT CT Patient Dosimetry Calculator, version 0.99x; ImPACT Group, London, England, <http://www.impactscan.org/ctdosimetry.htm>) (23) and was multiplied by pediatric normalised values (24).

Statistical analysis

The Wilcoxon signed rank test was used to evaluate the medians of the end-inspiratory and end-expiratory CT scores. Intraclass correlation coefficients (ICC) and Bland-Altman plots were used to evaluate agreement between both Brody-II scores and to assess the inter- and intraobserver agreement. ICC values of 0.41–0.60 indicated moderate agreement; 0.61–0.80, good agreement; and 0.81 or greater, very good agreement. Spearman correlation coefficients (ρ) were used to investigate correlations between CT and plethysmographic volume estimates. Software (SPSS,

version 14.0 for Windows; SPSS, Chicago, Ill) was used for all analyses. Inferential tests were considered to be significant if the P value was less than 0.05.

Results

Study population

The cohort consisted of eight male patients (median age, 15.7 years; interquartile range, 11.2–18.7 years; range, 7.0–20.3 years) and 12 female patients (median age, 12.4 years; interquartile range, 10.8–13.5 years; range, 6.3–17.2 years). Patient characteristics are given in Table 1. Spirometry and body plethysmography were performed on the same day as CT in 19 children. One child was too young to perform pulmonary function tests. For end-inspiratory CT, the effective tube current–time product depended on tube voltage and patient size and ranged from 23 to 34 mAs. When using 80 and 110 kV, mean dose-length product for end-inspiratory CT was 2.6 and 3.2 times higher, respectively, than that for end-expiratory CT. Owing to the differences in scanning protocols, mean effective dose was 0.69 mSv for end-inspiratory CT and 0.35 mSv for end-expiratory CT. For a 13-year-old, the median age in this cohort, effective dose was 1.2 mSv for end-inspiratory CT and 0.4 mSv for end-expiratory CT.

Table 1. Baseline characteristics in 20 patients.

Characteristic	Datum
Sex*	
Male	8
Female	12
Age (y)	12.6 (11.2 – 15.8) [6.3 – 20.3]
Height (m)	1.55 (1.38-1.69) [1.23 – 1.78]
Weight (kg)	43 (31-54) [23 – 66]
Forced expiratory volume in 1 sec†	100 (78-108) [46 – 127]
Forced vital capacity†	99 (80-113) [61 – 123]
Brody-II total score‡	
Inspiratory scan	11 (7-24) [2 – 55]
Expiratory scan	12 (5-20) [2 – 52]

Note – Unless otherwise specified, data are medians, with interquartile ranges in parenthesis and ranges in square brackets.

* Data are number of patients.

† Data are percentages of predicted values.

‡ Data are percentages of maximum possible scores.

Volume level CT

The median lung volume calculated from end-inspiratory CT scans was 3.3 L (range, 2–6 L), corresponding to a median of 77% (range, 55%–106%) of the measured TLC. The median volume calculated from end-expiratory CT scans was 1.8 L (range, 0.6–3 L), which was a median of 86% (range, 49%–153%) of the measured FRC and 140% (range, 83%–293%) of the measured RV (Figure 1). Thus, end-expiratory CT was obtained at a lung volume closer to FRC than to RV. Strong correlations were observed between the volume calculated from end-inspiratory CT scans and TLC ($\rho=0.71$; $P=0.001$) and between the volume calculated from end-expiratory CT scans and FRC ($\rho=0.92$; $P=0.001$) and RV ($\rho=0.92$; $P=0.001$) (Figure 1).

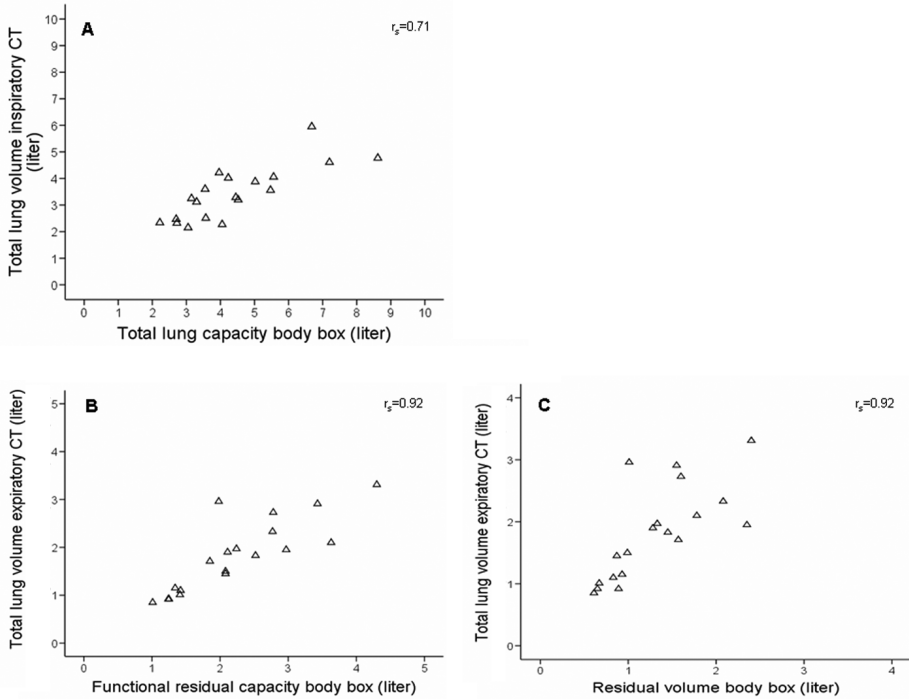


Figure 1. Plots show correlation between lung volumes calculated from CT scans and those measured with body box plethysmography. Plot A shows correlation between volume calculated from inspiratory CT scan and TLC. Plot B shows correlation between volume from expiratory CT scan and FRC. Plot C shows correlation between volume from expiratory CT scan and RV.

Inspiratory- versus expiratory CT scores

No significant differences were found between end-inspiratory and end-expiratory CT scans for Brody-II total ($P=0.776$) (Figure 2), bronchiectasis ($P=0.283$), airway wall thickening ($P=0.600$), mucus plugging ($P=0.070$), and opacities ($P=0.565$)

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scores. In addition, excellent agreement among all inspiratory and expiratory Brody-II scores was found (Table 2). Bland-Altman plots for the Brody-II total score (Figure 2), and each of the component scores showed that the differences between inspiratory and expiratory CT scores were independent of the magnitude of the scores. An illustration of matching structural changes on end-inspiratory and end-expiratory CT images is shown in Figure 3.

Intra- and interobserver agreement was good or very good for all Brody-II components (Table 3), and the corresponding Bland-Altman plots showed that variations in scores were independent of the magnitude of the scores.

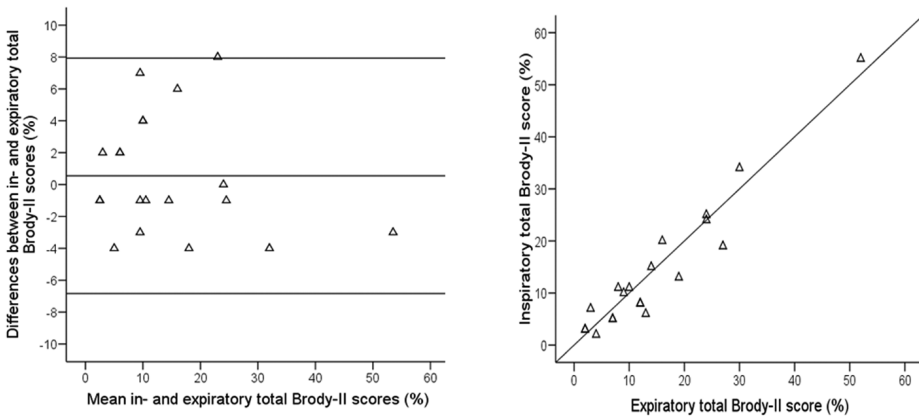


Figure 2. Two plots illustrating the correlation between the total Brody-II scores from inspiratory and expiratory CT scans. The Bland-Altman plot on the left shows that the difference in percentage between inspiratory and expiratory total Brody-II scores is independent of the magnitude of the scores. The lines in the plot represent the mean of the difference between the inspiratory and expiratory scores and the 95% upper and lower limits of agreement respectively. The plot on the right shows the correlation between inspiratory and expiratory total Brody-II scores.

Table 2. Intraclass correlation coefficient with the 95% confidence interval (CI) showing the agreement between inspiratory and expiratory Brody-II total and component scores.

Brody-II scoring system component	Intraclass correlation coefficient	95% CI
Brody-II total score	0.99	0.96 – 0.99
Bronchiectasis score	0.96	0.87 – 0.98
Airway wall thickening score	0.94	0.86 – 0.98
Mucus plugging score	0.98	0.94 – 0.99
Opacities score	0.91	0.78 – 0.96

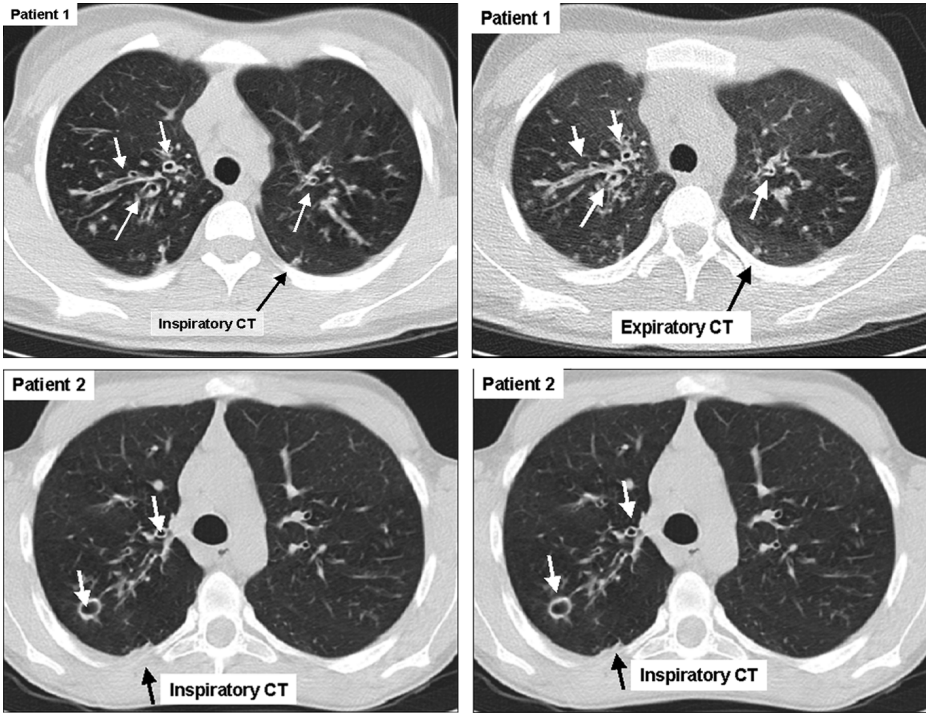


Figure 3. CT images of two patients show that structural changes can be accurately detected on both inspiratory and expiratory images obtained at the same level. Images for patients 1 and 2 showed lung volume that was 108% and 106%, respectively, of predicted TLC, while expiratory images showed 119% and 79%, respectively, of predicted FRC. Black arrows = small consolidations, white arrows = bronchiectasis.

Table 3. Intra- and interobserver agreement for Brody-II scores from inspiratory and expiratory CT scans.

Brody-II category	Intraobserver ICC		Interobserver ICC	
	Inspiratory CT	Expiratory CT	Inspiratory CT	Expiratory CT
Brody-II total score	0.97	0.98	0.90	0.82
Bronchiectasis score	0.98	0.97	0.84	0.76
Airway wall thickening score	0.94	0.97	0.75	0.70
Mucus plugging score	0.90	0.92	0.89	0.91
Opacities score	0.91	0.92	0.86	0.84

Discussion

In our study, end-inspiratory and end-expiratory CT scores matched closely, suggesting that end-expiratory CT alone may suffice for assessing CF-related structural abnormalities, thus allowing a reduction in radiation dose by up to 75%. We showed that diseased airways can be adequately identified on end-expiratory CT images. However, before we recommend this approach for monitoring patients with CF, a number of issues need to be addressed. First, our study was conducted in a cohort that did not include children younger than six years. Whether end-expiratory CT suffices for assessing structural changes in young children should be further investigated, ideally by using controlled-volume procedures (10). The most common previously reported structural abnormalities in young children are trapped air and bronchiectasis. For trapped air, end-expiratory CT is the optimal technique. For bronchiectasis, present in one-third of young children with CF, detection with volume-controlled end-inspiratory and end-expiratory CT images was shown to be substantially different(25). However, these results were found in children aged 1.4–3.6 years. Whether these results also apply to children older than 3.6 years is unknown and should be investigated.

Second, disease severity in our cohort ranged from no abnormalities to advanced disease. Only three patients with end-stage lung disease(26) were included. Clearly, this number is too small to prove that end-expiratory CT may suffice for end-stage lung disease in patients with CF. However, we do consider that it likely is sufficient because the structural abnormalities differ from those of patients with mild CF only in extent(27-28). Furthermore, trapped air (as measured by the RV/TLC ratio) is probably more extensive in end-stage lung disease, as it has been shown to increase with age(29). Clearly, this factor should be further investigated.

Third, it is unknown whether disease progression can be tracked on endexpiratory CT images with equal sensitivity as that that has been shown for endinspiratory CT images(5-6). To evaluate this factor, longitudinal end-expiratory CT scans need to be analyzed.

We investigated CT scans obtained with voluntary breath holds, which are used in most centers worldwide. Lung volume on inspiratory CT scans was shown to be close to the TLC, but lung volume on expiratory CT scans was clearly above the RV. However, we compared supine CT volumes with sitting plethysmographic volumes. Since the supine position leads to a reduction in lung volume(30), the reported differences between CT and plethysmography may have been smaller had similar postures been used. Spirometer-controlled CT scans help to standardize inflation levels and are highly reproducible(31), but involve a substantially more complicated procedure. The inspiration level is important, as it influences airway dimensions and the mechanical properties of healthy airways(32). At full

expiration, airways may be less distended than at inspiration, especially in young children(10). Little is known about the effect of airway disease on the mechanical properties of these airways. In adults, the diameter of bronchiectatic airways was reduced by 40% between voluntary breath-hold end-inspiratory and end-expiratory CT images(33). We observed that, on end-expiratory CT images obtained during voluntary breath holds, many bronchiectatic airways remained clearly visible and could be easily recognized as abnormal. This finding suggests that the physiologic reduction in airway diameter at expiration is impaired in bronchiectasis, thus allowing its identification on expiratory images. Additional studies will have to be performed to examine whether spirometer-controlled end-expiratory CT is also sensitive enough to detect bronchiectases. For trapped air, spirometer-controlled scans would be ideal, as scanning at lung volumes higher than the RV has been shown to overestimate the volume of trapped air(12, 18, 34). Whether the magnitude of this difference is of any relevance is unclear and should be investigated further.

The reduction in radiation dose when using only end-expiratory CT is substantial. Doses of 3 mSv per pediatric chest CT scan are not uncommon(35). In contrast, our end-expiratory CT dose was typically 0.4 mSv (110 kV), in agreement with reported values(18), which is approximately one-eighth of the annual U.S. background radiation dose of 3 mSv per person(35). Biannual CTs have been advocated to monitor onset and progression of CF lung disease (36). To minimize the lifelong risk of cancer inherent to frequent scanning (37), low-dose inspiratory protocols with a modulating-beam current and ultralow-dose expiratory protocols with a fixed-beam current were developed. Using only end-expiratory CT further reduces lifelong cancer risks related to our protocol to one-fourth of previous estimates(37-38), which were considered quite conservative. Therefore, monitoring structural CF-related changes by using only end-expiratory CT biannually is likely to be valuable and safe.

The six-section scanner we used is relatively old. For state-of-the-art multidetector scanners, the absolute dose values may be different, depending on the protocols used. However, we expect the relative dose reduction from using only end-expiratory CT with these modern scanners to be of similar magnitude. Radiation-free alternatives, such as chest magnetic resonance imaging, have been suggested, but their use for the assessment and follow-up of CF-related structural abnormalities needs to be further investigated before routine use can be recommended (39).

In conclusion, voluntary breath-hold end-inspiratory and end-expiratory CT scores matched closely in patients with mild CF, suggesting that end-expiratory CT may be sufficient to assess CF-related changes. However, larger studies that use volume-controlled CT imaging are needed before end-expiratory CT can be recommended as the optimal protocol for monitoring all patients with CF.

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Chapter 3

Trapped air assessment in CF
and the number of computed
tomography slices evaluated

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Abstract

Purpose To estimate the effect of the number of computed tomography (CT) slices on trapped air (TA) assessment in cystic fibrosis (CF) using an established and a new quantitative scoring system, and to compare CT and pulmonary function test (PFT) estimates of TA in a cross-sectional and longitudinal study.

Methods In this institutional review board approved pilot study, twenty children aged 6-20 years (12 girls, 8 boys, median age 12.6 years) contributed two expiratory CTs (3-slice CT₁, volumetric CT₂) and two PFTs (PFT₁, PFT₂) over two years after parental informed consent. From CT₂, 7 sets were composed; set 1 was volumetric, sets 2, 3, 4, 5, had a spacing of respectively 2.4, 4.8, 9.6, and 20.4 mm between slices. Set 6 and 7 contained 5 and 3 slices. Longitudinal follow-up was done with 3 slices. All scans were de-identified, randomized and TA was scored with the Brody-II system (CT_{TA(brody)}) and a new quantitative system (CT_{TA(quant)}). Analysis included Wilcoxon's sign test, Spearman's correlation (r_s), intra-class correlation coefficients (ICC) and linear mixed models.

Results For CT_{TA(brody)}, ICC for set 1 versus sets 2 to 7 was 0.75 to 0.87, but mean scores from set 6 and 7 were significantly lower than mean scores from set 1 ($p=0.01$ and $p<0.001$). For CT_{TA(quant)}, the number of slices did not affect TA assessment (ICC's 0.82 to 0.88, all $p>0.13$). CT and PFT estimates were not correlated ($r_s=-0.19$ to 0.09 , $p=0.43$ to 0.93). No change in TA over time was found for CT and PFTs (all $p>0.16$).

Conclusions The number of slices affected CT_{TA(brody)} estimates, suggesting that 3-slice protocols underestimate TA assessment in CF using the Brody-II system, CT and PFT estimates of TA showed no correlation, and no significant change over time.

Introduction

For patients with cystic fibrosis (CF), morbidity and mortality are mainly determined by the severity of lung disease. Trapped air (TA) is an early and important feature of CF lung disease (1-4), which occurs frequently. Studies showed that nearly two thirds of children aged 3 months showed signs of TA, even when asymptomatic (5). In addition, TA forms a substantial component of the abnormalities seen in patients with end stage CF lung disease (6). Furthermore, TA is increasingly recognised as an important endpoint for clinical trials (7). TA likely reflects peripheral airway pathology (8), and is traditionally determined by pulmonary function tests (PFTs). End-expiratory chest computed tomography (CT) is a more direct method to detect TA (1, 9-11). PFT estimates of TA (PFT_{TA}) have been shown to correlate with CT estimates of TA (CT_{TA}), however, CT_{TA} was thought to be more sensitive (9, 12-14). In addition, CT has the advantage over PFTs that it can assess both TA severity and distribution. To date, CT_{TA} is mainly estimated using semi-quantitative scoring systems (15). In these systems, pattern and/or severity of TA are assigned scores ranging from 0 to 3. A major disadvantage of this approach is that it is difficult to understand the clinical relevance of the scores. CT_{TA} estimates expressed as percentage of total lung volume would be easier to understand. An additional advantage of these estimates is that it allows a more straight forward comparison between CT_{TA} and PFT_{TA} . Few studies have investigated the longitudinal progression of TA using semi-quantitative CT scoring systems or PFTs. These studies show conflicting results. PFT studies have observed progression (16), no change (17) or improvement of TA over time (18), while CT studies all showed progression of TA (19-20).

To use CT for monitoring of TA, it is of key importance to minimise the radiation dose delivered by CT. Therefore, it is common practice to determine TA using only 3 expiratory slices (3, 5, 7, 19-24). However, it is unclear whether this approach is sensitive enough, since the minimal number of slices to compute CT_{TA} accurately has never been systematically studied in CF. A small study in 10 CF patients showed that accurate TA estimates could be obtained sampling 20 slices out of a volumetric CT (25). Another study in lung transplant recipients showed significant differences between CT_{TA} scores from protocols with 10 mm spacing between slices and scores derived from 3-slice protocols (26). This suggests that 3 slices may be insufficient for accurate CT_{TA} assessment. To test this hypothesis for CF patients, we performed this pilot study to estimate the effect of the number of CT slices on TA assessment in CF using an established and a new quantitative scoring system, and to compare CT and PFT estimates of TA in a cross-sectional and longitudinal study.

Materials and methods

Patient population

Twenty consecutive CF patients monitored at a single tertiary CF clinic were selected for this retrospective study. All CF patients at this institution undergo a routine annual assessment including biannual CT scans and annual PFTs. In this study, we included all patients that could contribute two of these routine CT scans and PFTs during annual check-up when clinically stable between March 2004 and August 2007. Clinical instability at the time of the assessment was the exclusion criterion. Eight children on intravenous antibiotics were considered unstable and were therefore not included. Institutional review board approval and informed consent for the retrospective use of anonymized data was obtained from the parents of all subjects.

Characteristics of the study cohort (12 girls, 8 boys) are shown in Table 1. For PFT₁, 3 children performed PFTs > 3 months of CT date and were thus excluded from the analyses, and 4 children were too young for testing. For PFT₂, all children performed PFTs within 3 months of CT date, except 1 child who was too young for testing.

Table 1. Baseline characteristics of the study cohort. The p value indicates the significance of differences in patient characteristics at the time of CT1 and CT2. Data given are patient numbers (%) or median (range). CT scores for CT2 are given for the 3 slice set only (set 7).

Characteristic	CT1	CT2	p value
Age (years)	10.6 (6 – 18)	12.6 (8 – 20)	*
BMI (kg/m ²)	16.2 (14 – 20)	17.3 (14 – 21)	0.002
FVC (% predicted)	97 (60 – 127)	99 (61 – 123)	0.28
FEV1 (% predicted)	97 (41 – 119)	100 (46 – 127)	0.85
FEF75 (% predicted)	58 (8-151)	69 (6-135)	0.74
Brody-II air trapping score (%)	16 (0 – 83)	20 (0 – 59)	0.53
Quantitative scores (%)	3 (0 – 46)	10 (0 – 34)	0.12
RV/TLC ratio (%)	27 (20 – 36)	28 (20 – 37)	0.18
TLCbb-TLChe/TLCbb (%)	9 (4 – 19)	13 (0 – 21)	0.95

* not tested

CT scans and evaluation

Each child contributed two voluntary breath hold expiratory CTs over two years. Scanning was done in supine position from apex to base using a 6-slice scanner (Somatom Emotion, Siemens Medical Solutions, Erlangen, Germany). Two scanning protocols were used. Baseline CT (CT₁) consisted of 3 slices taken at defined anatomical positions (Figure 1), using the following settings: rotation

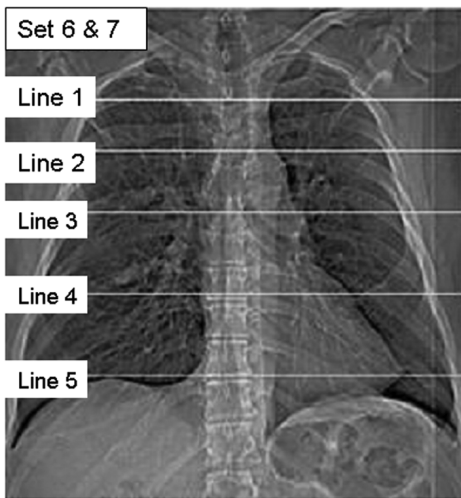


Figure 1. Predefined anatomical positions of the 5 respectively 3 images from which the CT_2 subsets 6 and 7 were composed. Line 1 represents the level between lung apex and top of the aortic arch (set 6&7), line 2 the top of the aortic arch (set 6), line 3 the position just below the carina (set 6&7), line 4 the level between carina and top of the diaphragm (set 6) and line 5 the top of the diaphragm (set 6&7). Lines 1, 3 and 5 also represent the levels of the images of the 3-slice CT_1 .

calculator (27), multiplied with pediatric normalised values (28)) for this protocol was 0.02 mSv. Follow up CT (CT_2) was volumetric (ultra low dose) using the following settings: rotation time 0.6-sec, tube voltage 80 or 110 kV (weight < or > 35 kg), pitch 1.5, 6x2 mm collimation, kernel B60s, and a fixed current of 25 mA, i.e. an effective tube current-time product of 10 mAs. Scans were reconstructed with a 2.5 mm slice thickness, and 1.2 mm increment. Mean radiation dose for the 20 scans made with this protocol was 0.4 mSv.

time 1.0-sec, tube voltage 110 kV, slice thickness 1.0 mm, kernel B70s, and a modulating current with a reference tube current-time product of 36 mAs. Mean radiation dose (calculated using the impact dosimetry

Effect of the number of slices on TA assessment

To estimate the effect of the number of slices on CT_{TA} assessment, 7 sets were composed from CT_2 . Set 1 contained all slices and was considered the “gold standard”. Set 2, 3, 4, and 5, had spacings of respectively 2.4, 4.8, 9.6, and 20.4 mm. These sets were generated by deleting respectively 1, 3, 7, 16 slices between the slices used for analysis. Set 6 and 7 contained 5 and 3 slices at predefined anatomical positions (Figure 1). All scans were scored with the Brody-II system ($CT_{TA(brody)}$), evaluating TA pattern and severity (21). Scores were expressed as percentage of the maximal possible score on a 0-100% scale. In addition, we developed a new quantitative scoring system to compute TA volume expressed as percentage of total lung volume ($CT_{TA(quant)}$, Figure 2). First, the lung tissue including regions of TA was automatically segmented (29), and total lung volume in milliliters was computed. Second, TA volume per slice was assessed using a digital 10x10mm grid and manually counting the cells projected over TA (30) (<http://mipav.cit.nih.gov/>). Partially filled cells were counted for the proportion of TA only. TA volume per slice was calculated by multiplying the number of grid cells by the grid cell volume in milliliters. Third, average CT_{TA} volume was computed by summing TA volumes (in milliliter) of each slice and dividing this by the total lung volume.

Scans were de-identified, randomized, and scored by a single observer (ML, 4 years of experience). This observer re-scored all CTs after 4 months to establish intraobserver variability. Mean scores from the first and second scoring session were used for analysis.

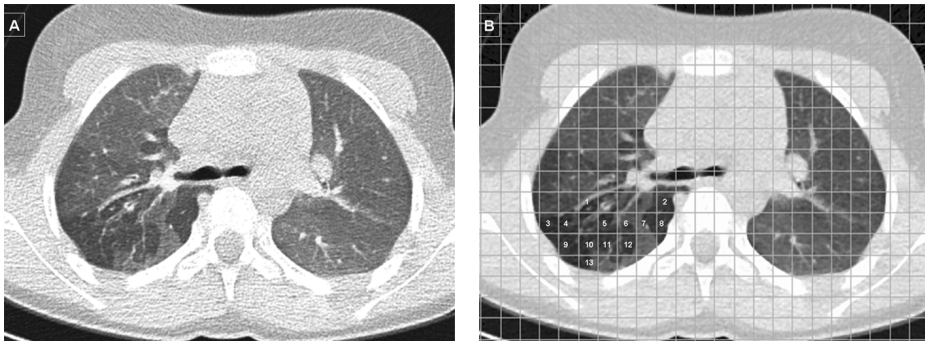


Figure 2. CT slices illustrating the new quantitative scoring system. First, the lung volume is automatically segmented (A), and the total lung volume in milliliters is computed. Second, TA volume per slice was assessed using a digital 10x10 mm grid and manually counting the number of cells projected over TA (B). TA volume per slice was then calculated by multiplying the number of TA grid cells by the volume of a grid cell in milliliters. Third, to compute average CT_{TA} volume for the complete scan, TA volumes (in milliliter) of each slice were summed and divided by the total lung volume of the complete scan. *A full color version of this image can be found on page 216 in the color section*

CT - PFT comparison and the course of TA over time

Lung volumes were obtained using a Masterlab Body Plethysmograph (Erich Jaeger AG, Würzburg, Germany) using the panting technique (31). Spirometry was done with a Jaeger diagnostic system (Erich Jaeger AG, Würzburg, Germany) following ERS guidelines. Reference values were according to Zapletal (32). PFTs were obtained within 3 months of CT_1 (PFT_{1TA}) and CT_2 (PFT_{2TA}), and excluded if otherwise. Used were: 1) ratio residual volume to total lung capacity (RV/TLC), and 2) the difference between TLC from body plethysmography (TLC_{bb}) and TLC from helium dilution (TLC_{he}) expressed as percentage ($(TLC_{bb} - TLC_{he})/TLC_{bb} * 100\%$). Cross-sectionally, we only compared PFT_{2TA} and CT_{2TA} estimates of set 1, as these were considered most precise in the assessment of TA. Longitudinally, we compared the 3-slice CT_1 (CT_{1TA}) with the 3-slice set of CT_2 (CT_{2TA}), and PFT_{1TA} with PFT_{2TA} .

Statistical analysis

Intraclass correlation coefficients (ICC) were used to investigate agreement of $CT_{TA(brody)}$ and $CT_{TA(quant)}$ between sets 1 versus sets 2 to 7, and to assess intraobserver variability. Although no universally applicable standards are available for what constitutes poor, fair or good reliability, ICC between 0.4 and 0.6, 0.6 and 0.8 and ³ 0.80 are generally considered to represent moderate, good and very good agree-

ment, respectively. For $CT_{TA(brody)}$ and $CT_{TA(quant)}$ separately, mixed model Anova with the Dunnett method to adjust for multiple comparisons was used to test for differences between the assumed “gold standard” set 1 and sets 2 to 7. Wilcoxon signed rank test was used to test for differences in patient characteristics at the time of CT_1 and CT_2 , and to evaluate the course of PFT_{TA} and CT_{TA} over time. Spearman’s correlation coefficient (r_s) was used to correlate PFT_{TA} and CT_{TA} . SPSS version 15.0 for Windows and SAS version 9.2 was used for analyses. Results are displayed as median (range) unless defined otherwise. $P < 0.05$ was considered significant.

Results

Effect of the number of CT slices on TA assessment

The respective mean number of slices for CT_2 sets 1 through 7 was 187, 82, 36, 19, 12, 5, and 3. Median scores per set are shown in Figure 3. Intraobserver variability for $CT_{TA(brody)}$ and $CT_{TA(quant)}$ scores for the sets was good with respective ICC values of 0.75 and 0.76 (set 1), 0.74 and 0.64 (set 2), 0.68 and 0.70 (set 3), 0.73 and 0.74 (set 4), 0.66 and 0.67 (set 5), 0.66 and 0.85 (set 6), and 0.60 and 0.85 (set 7). The mixed model Anova showed significant differences between mean $CT_{TA(brody)}$ scores from set 1 and mean scores from set 6 and 7 (Table 2). These mean scores were respectively 7% and 10% lower than scores from set 1. The ICC showed good agreement between set 1 and all other sets (Table 3). For $CT_{TA(quant)}$ scores, no significant differences were found between set 1 and the other sets regarding mean levels (Table 2). Good agreement was present between set 1 and the other sets for this score (Table 3).

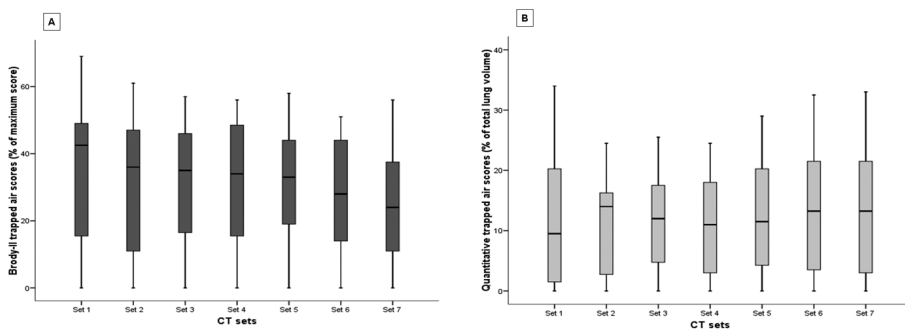


Figure 3. Boxplots showing the distribution over the 7 sets of CT_2 for the Brody-II scores in percentage of maximum score (A) and the quantitative scores in percentage of total lung volume (B). Boxes represent the interquartile range, the bars in the boxes display the medians. Minimal and maximal values are also shown.

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Table 2. The mean value for set 1, and the mean differences (adjusted p values) for pairwise comparisons of average CT trapped air scores of set 1 versus sets 2-7 for the Brody-II system and the quantitative system.

Brody-II scoring system						
Set 1	2	3	4	5	6	7
mean						
34.5	-1.9 (0.92)	-4.5 (0.22)	-4.1 (0.30)	-5.1 (0.13)	-7.2 (0.01)	-9.6 (<0.001)
Quantitative scoring system						
Set 1	2	3	4	5	6	7
mean						
11.8	-1.4 (0.72)	-0.6 (0.99)	-1.3 (0.79)	1.1 (0.90)	2.6 (0.13)	1.8 (0.46)

Table 3. Intraclass correlation coefficients (ICC) indicating the agreement between trapped air scores of the volumetric CT₂ and the scores of sets 2-7 for the Brody-II system and the quantitative scoring system.

Comparison	Brody-II system	Quantitative system
Set 1 vs 2	0.82	0.84
Set 1 vs 3	0.86	0.82
Set 1 vs 4	0.87	0.87
Set 1 vs 5	0.80	0.86
Set 1 vs 6	0.82	0.88
Set 1 vs 7	0.75	0.87

CT - PFT comparison and the course of TA over time

Cross-sectional analysis showed no correlation between follow up PFT_{TA} parameters RV/TLC and $(TLC_{bb} - TLC_{he})/TLC_{bb}$ and follow up CT_{TA(brody)} scores ($r_s=0.09$, $p=0.92$ and $r_s=-0.11$, $p=0.93$ respectively) and follow up CT_{TA(quant)} scores of set 1 ($r_s=0.03$, $p=0.90$ and ($r_s=-0.19$, $p=0.43$ respectively). RV/TLC was also not correlated with $(TLC_{bb} - TLC_{he})/TLC_{bb}$ ($p=0.34$).

Longitudinal analysis of CT_{TA} (3 slices) and PFT_{TA} did not show a significant change over two years: baseline and follow up estimates were not significantly different for CT_{TA(brody)} ($p=0.16$), CT_{TA(quant)} ($p=0.10$), RV/TLC ($p=0.63$), and $(TLC_{bb} - TLC_{he})/TLC_{bb}$ ($p=0.50$, Figure 4).

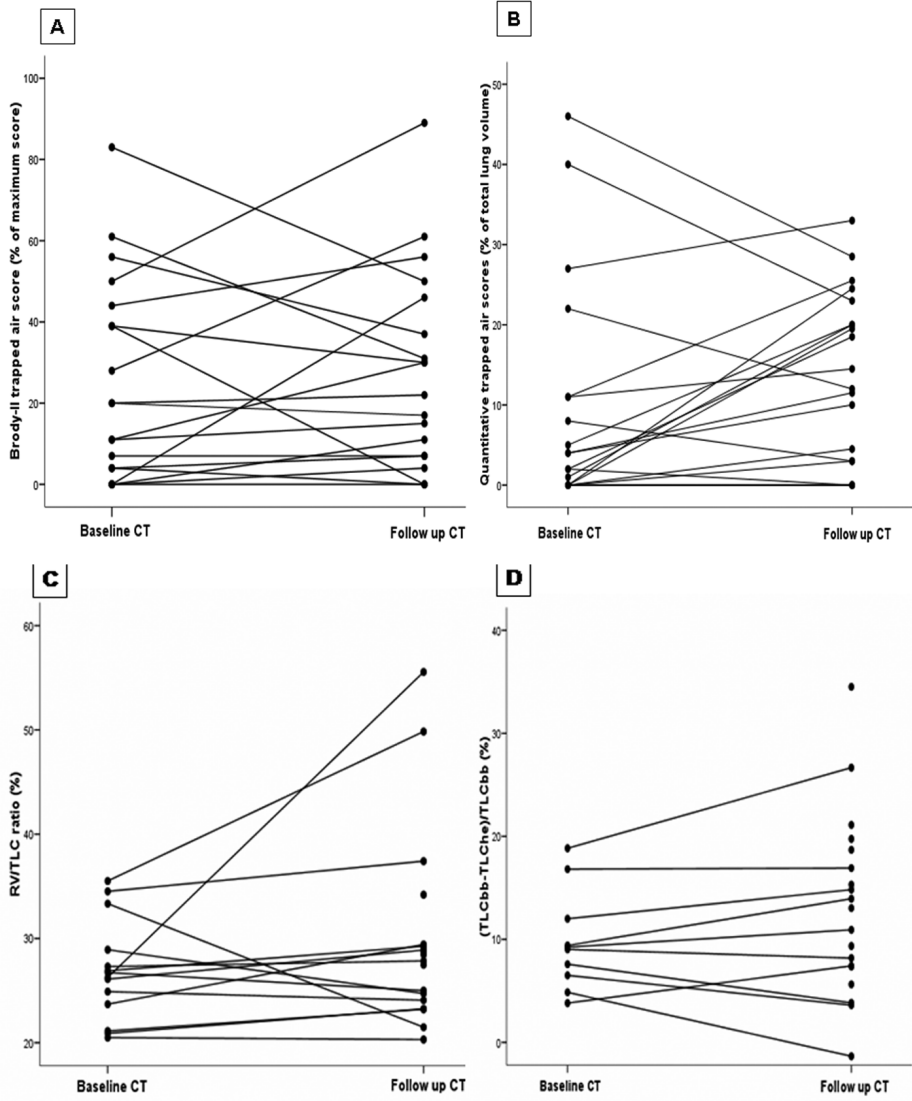


Figure 4. Charts illustrating the course of trapped air over time for individual patients. Plots A and B represent the Brody II (A) and the quantitative (B) trapped air scores over time, plots C and D indicate the pulmonary function test (PFT) estimates of trapped air over time. CT scores and PFT estimates of trapped air between the baseline and follow up CT were not significantly different.

Discussion

In this study, we aimed to estimate the effect of the number of CT slices on TA assessment in CF, using quantitative and Brody-II scores. In addition, we compared CT_{TA} and PFT_{TA} in a cross-sectional and longitudinal study. For both scoring systems, good agreement was found between sets 2-7 and the “gold standard” set 1, suggesting that there is no effect of the number of slices on the assessment of TA. However, there was a statistically significant difference between mean Brody-II scores from set 1 and mean scores from sets 6 and 7. This difference was in the order of 7% (set 6) and 10% (set 7). We considered the 10% difference to be substantial. This may imply that currently used 3-slice protocols underestimate TA using Brody-II scores. The hypothesis that 3-slice CT scans are not sufficient for TA assessment is supported by the results of 2 other studies. Goris and co-workers studied the effect of sampling density on TA assessment in CF, using volumetric CTs and automated TA scores. They found that the precision of TA measurements decreased markedly below a 3.4% sampling density (approximately 6-7 slices) (25). Bankier and co-workers studied the effect of reducing the number of CT images on TA assessment in lung transplant recipients using a 5-point scoring system. They found significant differences in scores from CTs with 10 mm spacing and simulated protocols with 20 mm spacing, and respectively 3, 2 and 1-slices (26). In contrast with these results is the finding that $CT_{TA(quant)}$ scores from set 1 and scores from sets 6 and 7 were not significantly different. Thus, the quantitative scoring system appears to be less sensitive to the effect of limited slice protocols. This suggests that the effect of the number of slices depends on the type of scoring system used. This needs to be further investigated.

Assessing the effect of the number of slices on TA assessment is not only important to minimize radiation, it also provides relevant information for clinical studies. In pilot studies in CF and asthma, CT_{TA} has been used as an endpoint (33-34). Our findings can be used for protocol design, and sample size estimation.

In this study, we used two scoring systems to quantify TA on CT. Our newly developed quantitative system has several advantages over the Brody system. First, $CT_{TA(quant)}$ is derived from automated lung volume estimates combined with simply counting grid cells, while $CT_{TA(brody)}$ requires recognition of TA patterns. Second, $CT_{TA(quant)}$ is a volume estimate, and clinically easier to understand than semi-quantitative $CT_{TA(brody)}$ scores. A disadvantage however, is its time consuming nature. Quantitative scoring for set 5 required 20 minutes, while Brody scoring required 5-10 minutes. However, the quantitative approach may be automated, reducing analysis time. Such systems have been developed (25), but, to our knowledge, are not yet commercially available.

In this study, CT_{TA} and PFT_{TA} were not significantly correlated. This is in contrast with other studies that did show a correlation between CT_{TA} scores and RV/TLC (9, 12). These differences could be due to differences in CT analysis (automated analysis systems versus our manual scoring systems), CT protocol (6 slices versus our volumetric protocol), or breath hold technique (spirometer-triggered versus our voluntary breath hold CT). However, we consider it more likely that PFTs may not estimate TA as precise as CT. PFT_{TA} is derived from body plethysmography, a measurement with relatively high variability (35). First, plethysmography tends to overestimate TLC, as it includes gas in nose, mouth, oesophagus and abdomen. Second, the variability between repeated measurements is high, with reported 95% limits of agreement (expressed as percentage of the mean of 2 measurements) of 8% for TLC and 40% for RV in healthy subjects (36). This variability increases with more severe airflow obstruction (35). Third, plethysmography has a number of potential error sources and requires constant conditions for temperature, pressure, atmosphere, and humidity.

CT is likely to be more sensitive to detect TA than PFTs. First, CT studies have shown that CT_{TA} could distinguish between CF patients and healthy subjects in infants (3) and older children (9, 12). This in contrast to PFT studies in which PFT_{TA} could not distinguish between patients and controls in infants (4), while showing contrasting results in older children (9, 12). Second, CT can detect TA early. Infant CT studies have been described using either free breathing (1-3, 7) or controlled-volume techniques (1-3, 7) to acquire the expiratory images needed to assess TA. Infant TA estimations using multiple breath washout techniques have been described (37-38), but the accuracy of this method and its value as an indicator of peripheral airway pathology needs to be further investigated. Standard PFT_{TA} estimates require patient cooperation, which is usually obtained around age 6 years. Third, CT can assess patterns of TA, allowing sensitive monitoring of its distribution over time. Thus, CT is likely to be more sensitive for TA detection than plethysmography. Additional studies are needed to investigate this further.

We observed no progression of CT_{TA} . This is in contrast to other CT studies that did show progression of TA over time (19-20). This could be due to differences in age of the study subjects, or the scoring method used. Alternatively, this finding could be due to the small sample size in our cohort. However, we consider it likely that 3-slice protocols, which are commonly used for patient care and clinical studies (3, 5, 7, 19-24), may not be sensitive enough to detect progression. The sensitivity to detect changes in TA over time can be improved by increasing the number of slices and by optimizing volume control during scanning.

Limitations of this pilot study are the small sample size, and the lack of volume control during CT scanning. This may have reduced the sensitivity to detect progression. Inspiration level is important, as CT scans near functional residual ca-

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capacity may overestimate TA relative to scans near RV (12). Volume-controlled scans could address this problem.

In conclusion, this pilot study suggests that expiratory 3-slice protocols underestimate TA in CF using the Brody-II system. PFT_{TA} and CT_{TA} showed no correlation. CT can assess both TA pattern and severity, and is likely to be more sensitive for early TA detection and follow up than PFTs. Additional studies are needed to investigate this further.

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Part 2

Image analysis

Chapter 4

The spectrum of structural abnormalities on CT scans from CF patients with severe advanced lung disease.

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Abstract

Rationale In cystic fibrosis (CF), lung disease is the predominant cause of morbidity and mortality. Little is known about the spectrum of structural abnormalities on computed tomography (CT) scans from CF patients with severe advanced lung disease (SALD). No specific CT scoring system for SALD is available.

Objectives To design a quantitative CT scoring system for SALD, to determine the spectrum of structural abnormalities in patients with SALD, and to correlate the SALD system with an existing scoring system for mild CF lung disease and pulmonary function tests (PFTs).

Methods 57 patients with CF contributed one CT made during screening for lung transplantation. For the SALD system, lung tissue was divided into four components: infection/inflammation (including bronchiectasis, airway wall thickening, mucus and consolidations) air trapping/hypoperfusion, bulla/cysts, and normal/hyperperfused tissue. The volume proportion of the components was estimated on a 0-100% scale; mean volumes for the whole lung were computed. Scores were correlated with Brody-II scores and PFTs.

Results The SALD system identified a wide spectrum of structural abnormalities ranging from predominantly infection/inflammation to predominantly air trapping/hypoperfusion. SALD infection/inflammation scores correlated with Brody-II scores (r_s 0.36 to 0.64) and SALD normal/hyperperfusion scores correlated with FEV₁ (r_s = 0.37). Reproducibility for both systems was good.

Conclusions A CT scoring system was developed to characterise the structural abnormalities in patients with SALD. A wide spectrum was observed in SALD, ranging from predominantly air trapping to predominantly infection/inflammation-related changes. This spectrum may have clinical implications for patients with SALD.

Introduction

Since the first description of cystic fibrosis (CF) in 1938, patients' life expectancy has greatly improved. Thanks to better treatment that curbs progression of pulmonary disease (1, 2), life expectancy is now around 40 years (3), and over 40% of CF patients are adults (4). Nevertheless, most patients still develop Severe Advanced Lung Disease (SALD), the predominant cause of mortality in CF.

Little is known about the structural abnormalities in SALD, as few pathology studies are available. These studies used lung specimens from transplant and/or autopsy procedures to describe the structural changes in SALD (5-7). To the best of our knowledge, no systematic studies have investigated SALD in vivo. Routine chest computed tomography (CT) scans from CF patients made for screening for lung transplantation may be used for this purpose. Knowledge on the structural changes in SALD is important, as it may indicate which structural abnormalities in CF lung disease can lead to SALD and hence, have to be monitored and treated in patients with early disease to prevent progression to SALD. Furthermore, it may give more insight in clinical differences and outcomes in patients with SALD. When SALD has established, lung transplantation is often the only treatment option left. To date, it has been a major challenge to determine which SALD patients have the highest risk of dying, and are thus most in need of a lung transplant. This is reflected in reported mortality estimates for patients awaiting transplantation, which range from 15% to 40% (8-10). Currently used prediction models for waiting list survival in these patients include clinical parameters, but no information on lung structure. CT may add important information to these prediction models, as it was proven to be more sensitive to detect and monitor CF lung disease than pulmonary function tests (PFTs) (1, 11-13). We speculate that the patient's clinical outcome may be impacted by the type of structural lung abnormality observed on CT. Our hypothesis is that, based on our clinical impression, a spectrum of abnormalities can be observed in SALD, ranging from predominantly infection/inflammation-associated changes such as consolidations and bronchiectases to hypoventilation-associated changes such as air trapping and hypoperfusion.

To test this hypothesis, a scoring method is needed to quantify the structural abnormalities in SALD in a systematic, objective and time-efficient fashion. Current scoring systems, such as the Brody-II system, are reproducible (14), but were primarily designed to quantify early and moderately advanced disease (11, 15, 16). For the CT scans of patients with SALD, a dedicated SALD scoring system may be more sensitive to detect differences in disease spectrum between patients.

Therefore, we aimed to 1) design a CT scoring system for the CT abnormalities of patients with SALD; 2) correlate this new system with the Brody-II system and

PFTs, and 3) investigate the spectrum of structural abnormalities on CT scans of patients with CF who have SALD.

Methods

Study population

In this retrospective study, data from patients with a confirmed diagnosis of CF and screened for lung transplantation between 2001 and 2005 was collected in three transplant centers. Patients were only included when screening data, including a chest CT scan, was available. Patient characteristics are defined in the online supplement. Screening criteria were based on internationally used recommendations (8, 17-20), although one center (center 3 in the analysis) used a forced expiratory capacity in 1 second (FEV_1) of $< 25\%$ for males and $FEV_1 < 40\%$ for females. The review boards of all three participating centers approved the study protocol and waived informed consent.

CT scanning procedures and scoring

Lung structure was evaluated with CT scans. Eight CT scanners (characteristics in online supplement) were used in this study. CT scans were anonymised before evaluation and analysed in random order. A single experienced observer scored all scans using the Brody-II scoring system (11) and a newly developed SALD scoring system. Reproducibility within and between observers was determined for both systems. Within observer agreement was tested by re-scoring a random subset of 25 scans. For between-observer agreement analysis, an independent experienced second observer scored a random subset of 25 scans. Both observers were blinded for clinical data and outcome of the patients.

Brody-II scoring system

This system evaluates bronchiectasis, airway wall thickening, mucus plugging and opacities on inspiratory images and air trapping on expiratory images (11). As expiratory images were lacking in 45/57 patients, the maximal possible total Brody-II score (207 points) was reduced by the air trapping score (27 points), thus changing the upper limit to 180 points. To enable direct comparison, scores were recalculated and expressed as percentages of the maximal possible score on a scale of 0 (no disease) to 100 (maximal lung disease).

SALD scoring system

The development of the SALD system is described in the online supplement. In brief, the SALD score aims to divide the total lung volume into 4 mutually-exclusive and comprehensive components of lung morphology, each assessed on a 0-100% scale. Three components indicate abnormalities: 1) infection/inflammation, which includes bronchiectasis, airway wall thickening, mucus and consoli-

datations, 2) air trapping/hypoperfusion, and 3) bulla/cysts. The fourth category, normal/hyperperfused tissue, reflects parenchyma that is normal or hyperperfused due to a redistribution of blood caused by perfusion defects. This tissue is still thought to contribute to normal gas exchange. For all CT slices (1 slice per 10 mm), the observer estimated the percentage of total lung area to be assigned to each component. Then, for each component separately, the volume estimates from all slices were summated and the sum was divided by the number of slices to obtain mean volume estimates. High scores for the first three categories reflect a high volume of structurally changed lung tissue and thus, severe disease. A high score for the normal/hyperperfusion component reflects a high volume of relatively normal lung tissue. Thus, in the SALD system, all lung tissue was assigned to one or more of the four SALD components, with these four component scores adding up to 100%. Therefore, the SALD scoring system consists of only four component scores and does not compute a total score.

Statistical analysis

For continuous and categorical variables, the Kruskal-Wallis and Chi-square test were used in the comparison of baseline characteristics between the centres. Correlations between SALD and Brody-II score and between CT scores and PFTs were investigated using Spearman's correlation coefficients (r_s). Reproducibility for both scoring systems was evaluated using intraclass-correlation coefficients (ICC) and Bland-Altman plots. Although no universally applicable standards are available for what constitutes poor, fair or good reliability (21), we considered ICC values between 0.4 and 0.6, 0.6 and 0.8, and 0.80 or greater to represent moderate, good and very good agreement. SPSS version 14.0 for Windows was used for all statistical analyses. Results are displayed as median (range) unless defined otherwise. A p value of <0.05 was considered significant.

Results

Study population

Data was collected from 57 consecutive patients. No significant differences in patient characteristics were observed between the centers, except for some components of the Brody-II system (Table 1). SALD component scores for bulla/cysts were excluded in further analyses, since this item was only present in 11/57 (19%) patients.

Part 2 Image analysis

Table 1. Patient characteristics and CT scores for the study cohorts in the three transplant centres.

Characteristic	Center 1	Center 2	Center 3	Total
N	10	12	35	57
Males	5 (50%)	8 (67%)	21 (60%)	34 (60%)
Age (years)	28.5 (16-38)	32.2 (16-49)	24.4 (17-53)	26.7 (16-53)
BMI (kg/m ²)	19.5 (16-26)	20.0 (18-22)	19.0 (15-27)	19.0 (15-27)
Pancreatic insufficiency	8 (80%)	10 (83%)	34 (97%)	52 (91%)
Diabetes Mellitus	5 (50%)	3 (25%)	9 (26%)	17 (30%)
Microbiology				
P. aeruginosa	10 (100%)	11 (92%)	32 (92%)	53 (93%)
B. cepacia Complex	0	2 (17%)	2 (6%)	4 (7%)
FEV ₁ (% predicted)	24 (19-34)	26 (15-38)	27 (13-45)	26 (13-45)
FVC (% predicted)	43 (25-70)	42 (29-67)	43 (24-89)	42 (24-89)
Brody-II scores				
Total score	40 (32-60)	48 (33-59)	34 (17-52)	37 (17-60)
Bronchiectasis	44 (29-57)	60 (35-72)	36 (25-60)	41 (25-72)
Mucus plugging	31 (25-47)	16 (8-42)	19 (0-42)	25 (0-47)
Airway wall thickening	31 (19-63)	54 (26-69)	35 (15-60)	36 (15-69)
Opacities	11 (6-26)	15 (7-26)	9 (0-22)	11 (0-26)
SALD scores				
Infection/inflammation	24 (17-30)	23 (17-41)	23 (9-43)	24 (9-43)
Air trapping/ hypoperfusion	48 (27-61)	36 (28-68)	43 (24-61)	43 (24-68)
Bulla/cysts	0 (0-11)	4.5 (0-39)	0 (0-13)	0 (0-39)
Normal/ hyperperfusion	29 (21-43)	31 (10-46)	31 (20-51)	30 (10-51)

Data are given as patient numbers (%) or as median (range).

BMI, Body Mass Index; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity;

SALD, severe advanced lung disease

CT scoring systems

Between and within observer agreement

Between and within observer agreement for both scoring systems was good, with most ICC values near or above 0.80 (Table 2). Bland-Altman plots showed that differences between the observers were independent of the magnitude of the scores in either scoring system (online supplement).

Table 2. Between and within observer agreement expressed as intraclass correlation coefficients for the Brody-II and SALD scoring system.

Type of scoring system	Within observer agreement	Between observer agreement
Brody-II scoring system		
Total score	0.77	0.80
Bronchiectasis	0.79	0.65
Mucus plugging	0.77	0.79
Airway wall thickening	0.56	0.73
Opacities	0.77	0.61
SALD scoring system		
Infection/inflammation	0.89	0.77
Air trapping/hypoperfusion	0.88	0.70
Bulla/cyst	0.99	0.98
Normal/hyperperfusion	0.71	0.68

SALD, severe advanced lung disease

SALD spectrum

Although all scans showed the SALD components infection/inflammation, air trapping/hypoperfusion and normal/hyperperfusion, there was a striking difference in the extent in which these abnormalities were present (Figure 1 and E5 on-line). Thus, a SALD spectrum could be distinguished ranging from predominantly infection/inflammation to predominantly air trapping/hypoperfusion (Figure 2).

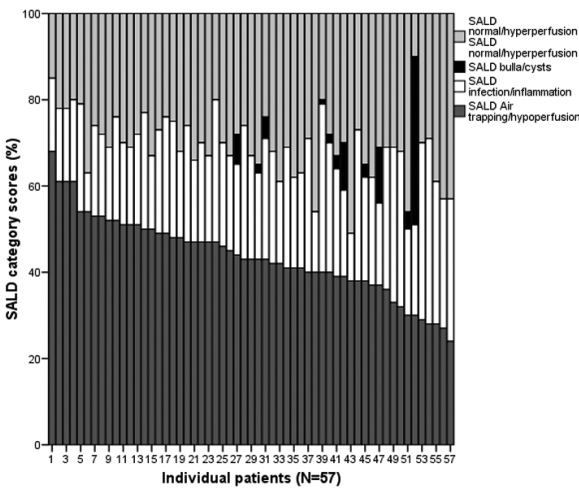


Figure 1. Distribution of the severe advanced lung disease (SALD) component scores. CT scans from lung transplant screening were scored according to the SALD criteria. A SALD spectrum was identified in which the dark grey bars represent the lung volume scored as hypoperfused tissue, the white bars infection/inflammation; the light grey bars normal/hyperperfused tissue; and the black bars bulla or cysts. Patients are sorted according to their air trapping/hypoperfusion component. The figure sorted for the infection/inflammation component can be found in the online supplement.

Part 2 Image analysis



SALD category scores	SALD category scores	SALD category scores
Infection/inflammation: 35%	Infection/inflammation: 15%	Infection/inflammation: 5%
Air trapping/hypoperfusion: 30%	Air trapping/hypoperfusion: 25%	Air trapping/hypoperfusion: 70%
Normal/hyperperfusion: 35%	Normal/hyperperfusion: 60%	Normal/hyperperfusion: 25%
Bulla/cysts: 0%	Bulla/cysts: 0%	Bulla/cysts: 0%

Figure 2. Illustration of the severe advanced lung disease (SALD) spectrum. Images show the spectrum of SALD-related changes in lung morphology and SALD component scores for each image. These range from infection/inflammation-related changes such as bronchiectasis (black arrows) to air trapping / hypoperfusion (white arrows). Image A shows predominantly bronchiectasis, image B shows a mix of bronchiectasis and air trapping / hypoperfusion, and image C displays minimal bronchiectasis but extensive air trapping and hypoperfusion.

Correlation SALD system – Brody-II system

Positive correlations were found between the SALD infection/inflammation score and the total Brody-II score ($r_s=0.64$ $p<0.001$; Figure 3) as well as with each of the Brody-II component scores: bronchiectasis ($r_s=0.59$ $p<0.001$), airway wall thickening ($r_s=0.62$ $p<0.001$), mucus plugging ($r_s=0.50$ $p<0.001$) and opacities ($r_s=0.36$ $p=0.006$). No significant correlations were found between the SALD normal/hyperperfusion score and the total Brody-II score or any of the component scores.

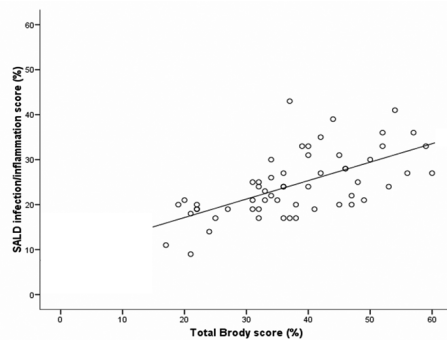


Figure 3 The correlation between the severe advanced lung disease (SALD) infection/inflammation score and the total Brody-II score.

Correlation CT scores – PFTs

Total Brody-II score correlated, albeit weakly, with forced vital capacity (FVC) ($r_s = -0.28$ $p=0.035$, Figure 4) but not with forced expiratory volume in 1 second

(FEV₁). None of the Brody-II component scores correlated with FEV₁, and only the component score airway wall thickening correlated with FVC ($r_s = -0.31$ $p=0.018$). None of the SALD components correlated with FVC, and only the normal/hyperperfusion score correlated with FEV₁ ($r_s = 0.37$ $p=0.005$, Figure 4).

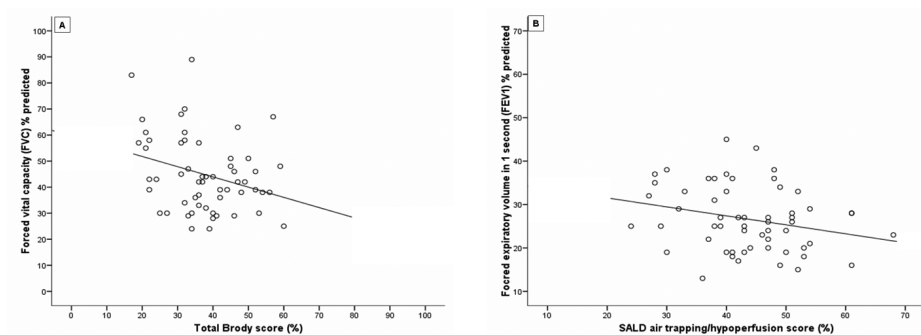


Figure 4. Correlation between CT scores and lung function parameters. Plot A shows the correlation between the total Brody-II score and the forced vital capacity (FVC), while plot B displays the correlation between the severe advanced lung disease (SALD) air trapping/hyoperfusion score and the forced expiratory volume in 1 second (FEV₁).

Discussion

To our knowledge, this is the first study in CF that systematically describes the structural abnormalities on CT scans from CF patients with SALD screened for lung transplantation. The most important finding of this study is the wide disease spectrum that was identified in patients with SALD in vivo, using the newly developed SALD scoring system. At one end of the spectrum, patients had predominantly infection/inflammation-related changes, and at the other end predominantly air trapping/hyoperfusion. The observed structural abnormalities have been described in pathology studies, which revealed the presence of inflammation, atelectasis, bronchiectasis, fibrosis, cyst formation, airway wall thickening, and a substantial loss of cartilage (5-7). In these pathology studies, it was well recognized that these abnormalities were unevenly distributed throughout the lung. However, whether substantial differences in disease spectrum between patients could be observed was not studied.

Infection/inflammation, which included bronchiectasis, was found to be an important disease component in SALD. The importance of bronchiectasis in CF has been well recognized (22-24). Hence, prevention of bronchiectasis is an important treatment target in patients with SALD. A striking observation is the finding that air trapping is another important disease component in many patients with SALD.

In some patients, it was clearly the predominant morphological substrate for their severely impaired lung function. Air trapping has been observed early in the disease process of CF (25, 26). In a small randomized controlled study, it was shown that treatment with dornase alpha in patients with mild to moderately severe CF lung disease reduced air trapping on CT and improved peripheral airway obstruction (27). These results suggest that air trapping may be reversible when treated early. Clearly, this warrants further investigation.

Our observation is not only important in terms of prevention of SALD, but can also be relevant for the management of patients with SALD. We feel that more tailored treatment of the subtypes in SALD at an earlier stage of the disease has the potential to reduce mortality and improve the quality of life. It is likely that the therapeutic strategy for SALD patients with predominantly bronchiectasis should be different from that of patients with predominantly air trapping. Whether air trapping in CF patients with SALD is reversible is unknown. To the best of our knowledge, no systematic therapeutic studies have been performed in CF patients with SALD with the aim to reduce the severity of air trapping. The effect of dornase alpha in CF patients with advanced disease has been studied, air trapping however was not included as an endpoint (28). This needs to be further investigated in clinical studies. In addition, we think that the CT information of SALD patients may improve patient selection for lung transplantation. Currently used selection criteria comprise predicted forced expiratory volume in 1 second (FEV_1) < 30%, rapid respiratory deterioration with predicted FEV_1 > 30%, $PaCO_2$ > 50 mmHg and/or PaO_2 < 55 mmHg on room air, and/or females <18 years of age with FEV_1 > 30% and rapid deterioration (8, 17-20). Several studies have aimed to identify better predictors of survival, but remarkably, CT related parameters were never evaluated (8, 17, 18). It has been suggested that patients with SALD and predominantly infection/inflammation-related changes on their CT have a poorer prognosis than patients with predominantly air trapping/hypoperfusion (29). If so, the SALD score infection/inflammation may be able to contribute to survival prediction models independent of lung function-related parameters. A large multi-center study is currently ongoing to investigate this further.

Correlating CT scores with PFT parameters revealed only one significant association, i.e. between the SALD air trapping/hypoperfusion score and FEV_1 . None of the Brody component scores correlated significantly with FEV_1 . A likely explanation is the limited range in FEV_1 in this cohort (from 13- to 45%-predicted), and / or the limited sample size. These correlations will be further investigated in our large multicenter study.

In this study, the reproducibility of the SALD scoring system in the evaluation of SALD-related structural abnormalities was comparable to that of the Brody-II scoring system. However, there are several reasons why we consider the SALD

scoring system to be more attractive for further development than the Brody score. First, the SALD system is probably easier to automate than the Brody system, as it is based on differentiation between areas with high density (infection/inflammation) and low density (air trapping). This in contrast to the Brody-II system, which is based on pattern recognition, and therefore difficult to automate. Automated analysis can likely further improve the SALD system's reproducibility. A challenge for the automated approach however, will be the range of CT scanners and scan protocols used in transplant centers, which likely affects density parameters. The semi-quantitative scoring systems used in this study are less sensitive for technical differences than currently available automated systems (30). A short term option to improve the precision of the SALD system is to use a digital grid to estimate the volume of the components, a method shown feasible for volumes of air trapping (31). Second, the SALD system is easier to learn than the Brody-II system. The latter requires estimating severity of lesions, has more components, and requires classifying abnormalities per lobe. Third, SALD scores are continuous variables representing the volume of abnormal lung tissue involved in infection/inflammation, air trapping, and normal tissue. Hence, it is easy to understand what the scores mean. This in contrast to the Brody scores, which are computed of scores for severity and extent of an abnormality. This makes it complicated to understand what the scores mean for the patient.

The development of an automated method for the SALD system is important. Currently, the most important drawback for the clinical use of the current SALD system is its time-consuming nature. The SALD system requires 45-60 minutes to score a single CT examination while the Brody-II system requires only 20 minutes. An automated approach can make the SALD scoring more time-efficient and therefore, more accessible for clinical use. Currently, we would recommend using the SALD scoring system solely to evaluate SALD CT scans. It provides insight into the predominant features of the abnormalities on the CT scans. This system has not yet been validated, however, for patients with mild to moderately advanced lung disease. Our next step, therefore, will be to further validate the SALD system in a large cohort and to study correlations between SALD scores and clinical outcome. In this analysis, we may include the observation of bullae/cysts in the air trapping/ hypofusion component, since this reflects lung tissue not contributing to gas exchange, and which likely shows little inflammatory changes.

This study has a few limitations. First, we used CTs that were obtained with eight different CT scanners and scanning protocols. This may have introduced some bias related to differences in resolution and density distribution. However, we consider it unlikely that this should have affected observation of the substantial differences in disease spectrum present in the patients. Before scoring, images were assessed on image resolution and movement artefacts. All were found to be of sufficient quality for scoring, with good reproducibility, so we may assume that the use of

different scanners was non-differential. In addition, manual semi-quantitative scoring systems generally are thought to be less sensitive to differences between CTs and protocols (30, 32). Second, the correlations between the components of the two scoring systems were limited by the absence of air trapping scores since expiratory scans were only available in 12/57 patients (21%). Evidently, expiratory images were not routinely included in the screening protocols before 2005. The absence of expiratory images likely had more impact on the Brody-II scores than on the SALD scores, as air trapping in the Brody system was completely excluded. The SALD component air trapping/hypoperfusion most likely included areas that would have been classified as air trapping on expiratory images. Third, we cannot be sure that the morphological features on CT adequately reflect the histology of these abnormalities. Several studies have shown correlations between CT morphology and histologic findings, although none of them included CF patients (33-35). However, a study in idiopathic pulmonary fibrosis patients showed that chronic cystic lesions, including bronchiectasis, correlated well with histology. This in contrast to ground glass opacities and consolidations on CT, that failed to correlate with histologic specimens (33). Additional correlative studies using CT scans and histology from CF patients could address this issue.

In summary, we designed a CT scoring system specifically for patients with CF who have SALD and tested this retrospectively on 57 CT scans made during screening for lung transplantation. The new SALD system is reproducible, and able to identify a wide spectrum of structural abnormalities in SALD. A striking finding was that air trapping/hypoperfusion was an important component of SALD, in addition to inflammation/infection (including bronchiectasis). Differences in disease spectrum may have implications for prognosis and treatment of CF patient with SALD. Our next step will be to link the SALD scores to clinical outcome, to determine the minimal important difference of changes in the component scores

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Patient characteristics

Diagnosis of CF followed from clinical CF features, positive sweat test, and/or the presence of two CF mutations. Age was defined as 'age at the time of the screening CT scan'. Pancreatic exocrine insufficiency was defined as maintenance treatment with pancreatic enzyme. Diabetes mellitus was defined by the use of subcutaneous insulin by the patient. This was obtained by chart review. A patient was considered chronically infected with a given micro-organism when it was cultured from three or more different sputum samples in the six months preceding screening. The following international used guidelines were used to determine the moment of screening for patients:

- Predicted forced expiratory volume in 1 second (FEV_1) < 30%
- Rapid respiratory deterioration with predicted FEV_1 > 30%
- $PaCO_2$ > 50 mmHg and/or PaO_2 < 55 mmHg on room air, and/or
- Females under the age of 18 years with FEV_1 > 30% and rapid deterioration (1-5).

Computed Tomography (CT) scanning protocols

Lung structure was evaluated using CT scans. In this multi centre study, 8 different CT scanners were used during the screening period.

In center one, three multi slice scanners were used (Sensation 16, Emotion 16 and Volume zoom, Siemens AG Medical Solutions, Forchheim, Germany). Scans were obtained using a beam current of maximally 390 mA, and dose modulation was used in two of the three scanners. The rotation time was 0.5-0.6 seconds, and the beam potential 110-120 kV. Scans were obtained from lung apex to base at intervals varying from 1.2-5.0 mm using 1.0-5.0 mm thick slices.

In center two, one single slice CT scanner (SR7000, Philips Medical Systems, Best, the Netherlands) and two multi-slice scanners (Brilliance 16 and the MX8000, Philips Medical Systems) were used throughout the study period. Scans were obtained using a beam current of 250 mA for the single slice scanner, and dose modulation was applied in the multi scanner protocol. The exposure time ranged from 0.5-1.0 seconds, and the beam potential was 120 kV for all three scanners. Scans were made from lung apex to lung base at 5-10 mm intervals using 1.0-5.0 mm thick slices.

In center three, a single slice CT scanner (Hi speed ZXi, GE Medical Systems, Milwaukee, WI, USA) was used up to October 2004, and a multislice CT scanner after October 2004 (Light Speed 16 Pro, GE Medical Systems). Scans were obtained using a beam current of 300-700 mA, a rotation time of 0.5-1.0s, and a beam potential of 140 kV from lung apex to base at 10 mm intervals using 1.25 mm thick slices.

Since different transplant centers use different scanners and scanning protocols, image quality was likely to vary. Therefore, the quality of the CTs was assessed before scoring. This was done by scrolling through the scan and determining whether the quality was sufficient for scoring. CT scans with severe movement artefacts were excluded from analysis.

CT scoring

Development of the SALD scoring system

In order to define tissue/morphology categories for use in the SALD scoring system, a panel consisting of a pediatric radiologist, a pediatric pulmonologist, and a PhD student systematically evaluated a random set of 10 CT scans acquired from CF patients during lung transplant screening. They classified the most prevalent structural abnormalities on these CT scans into 5 categories: 1) infection/inflammation; 2) air trapping and/or hypoperfused tissue; 3) hyperperfusion; 4) bullae or cysts, and 5) normal lung tissue, which formed the basis of the SALD scoring system.

Two independent observers tested this concept categorization on 10 CT's. This pilot indicated that clear distinction between areas of hyperperfusion areas of normal tissue was difficult to make. As the experts felt that hyperperfusion does not necessarily negatively influence lung function, these categories were combined into a single category 'normal/hyperperfusion' into which all tissue with functional gas exchange would fall. The final SALD scoring system therefore incorporated 4 categories — three components indicating abnormalities: 1) infection/inflammation, 2) air trapping/hypo perfusion, and 3) bulla/cysts; and one component reflecting tissue with a normal contribution to gas exchange: normal/hyperperfused tissue. The category infection/inflammation includes area with bronchiectasis, bronchial

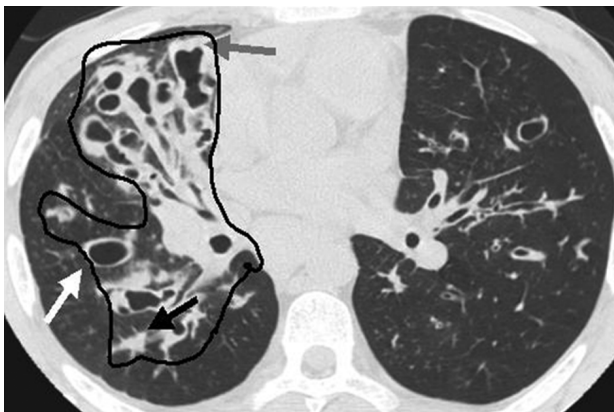


Figure E1. Illustration of the SALD category infection/inflammation. Shown is a CT scan of a CF patient with SALD. The areas in the right lung which would be scored as SALD 'infection/inflammation' are outlined in black and include abnormalities such as bronchiectasis (white arrow), bronchial wall thickening (grey arrow) and mucus plugging (black arrow).

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wall thickening, atelectasis, ground glass, consolidations, and mucus plugging (Figure E1). The definitions for these items are according to Brody et al (6).

The category air trapping/hypoperfusion (Figure E2) includes areas with a lower density than normal lung tissue, which are thought to represent poorly ventilated and hypoperfused parenchyma (7-9).

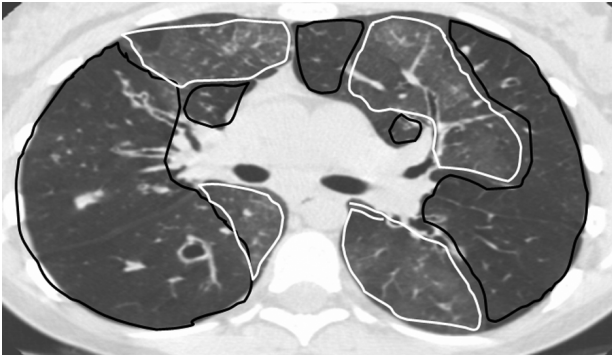


Figure E2. CT slice of a CF patient with SALD illustrating the SALD categories air trapping/hypoperfusion and normal/hyperperfusion. Areas which are hypodense due to air trapping and hypo perfusion are circled in black. Normal and hyperperfused lung parenchyma is circled in white.

The category bulla/cysts (Figure E3) represents areas of apparent parenchymal destruction thought to have no association with inflammation and no contribution to gas exchange. Both bulla and cysts are defined as more or less round, air-filled parenchymal spaces with well-defined walls and a diameter of more than 1 cm. Neither has an identifiable connection to the bronchial tree. A cyst has a wall thickness of more than 1 mm and a bulla of less than 1 mm (10).

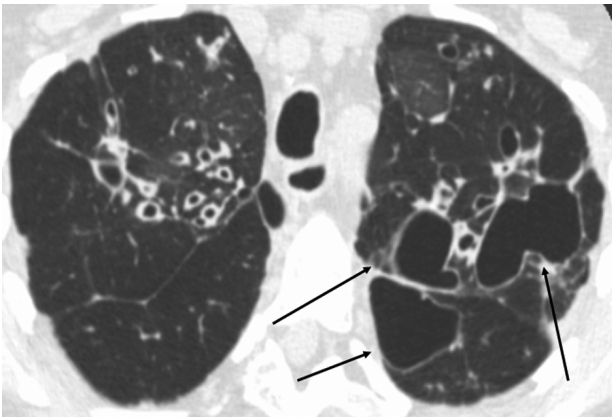


Figure E3. CT image to illustrate the SALD category cysts/bulla. Shown is a CT scan of a CF patient with SALD. The arrows indicate the bullae in the left lung of this patient.

The last category covers areas likely to contribute to gas exchange, including normal and hyperperfused tissue (Figure E2). Hyperperfusion appears on CT as areas with a higher density than normal lung parenchyma, and is considered a secondary effect related to areas of hypoperfusion.

After definition of the categories, we decided on a scoring system based on relative volume, and tested 2 methods to estimate the relative volume, each on 25 scans using 2 independent observers with respectively 1 and 4 years experience in scoring chest CT scans. For both methods the left and right lung were scored separately, and for both methods, all lung tissue was completely and exclusively divided over the SALD categories, so that the component scores added up to 100% by definition.

In the first method, the observer scrolls through the entire lung using all available slices and then directly estimates the volume of tissue in each category for the entire lung (the “scroll and score method”). In the second method, the observer estimates the percentage lung area in each category on each individual slice, which are averaged to determine the final SALD component score (the “single slice method”). For the single-slice method, we scored one image from each 10 mm interval; hence, not all available slices were scored. In the case of scans using a 10-mm interval, we did score all slices, while only every second slice was scored in scans using a 5-mm interval, and so on. Each image was scored independently and in random order. The precision level of each method was investigated by establishing the means and standard error of the means for each component. The Wilcoxon signed rank test was used to compare the methods. The single slice method was more precise and better reproducible. Therefore, only results obtained by that method are displayed in the results section.

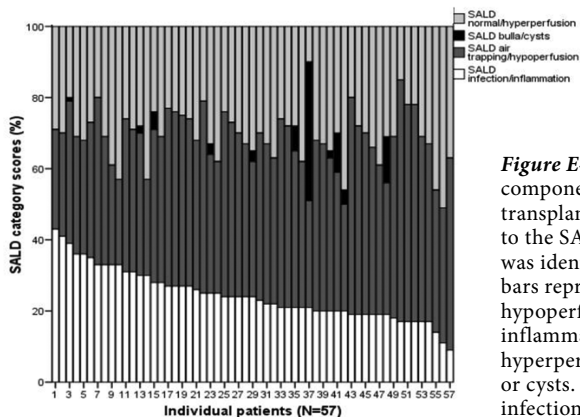


Figure E4. Distribution of the SALD component scores. CT scans from lung transplant screening were scored according to the SALD criteria. A SALD spectrum was identified in which the dark grey bars represent the lung volume scored as hypoperfused tissue, the white bars infection/inflammation; the light grey bars normal/hyperperfused tissue; and the black bars bulla or cysts. Patients are sorted according to their infection/inflammation component.

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Part 3

Further validating CT as
surrogate endpoint in CF

Chapter 5

Reversibility of trapped air on chest computed tomography scans of patients with cystic fibrosis, an automated approach

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Submitted to Radiology

Abstract

Rationale Trapped air (TA) is an important early change in cystic fibrosis (CF) lung disease, and can be determined using pulmonary function tests (TA_{PFT}) or computed tomography (TA_{CT}). Little is known about the course and reversibility of TA_{CT} over time, and how TA_{CT} compares with TA_{PFT} .

Aims To investigate changes in TA_{CT} volume and distribution over time, and to compare TA_{CT} with TA_{PFT} .

Methods In this institutional review board approved study, 30 consecutive children gave informed consent and contributed two CTs and PFTs over two years. TA_{CT} was determined using image analysis software. Localized changes in TA_{CT} were assessed by deforming CT_2 to match CT_1 , and measuring the relative volume of stable TA (TA_{stable}), disappeared TA ($TA_{disappeared}$) and new TA (TA_{new}). For TA_{PFT} we used the difference between TLC measured by plethysmography en helium dilution ($(TLC_{bb} - TLC_{he})/TLC_{bb}$), residual volume to total lung capacity ratio (RV/TLC), forced expiratory flow at 75% of vital capacity (FEF_{75}) and maximum mid-expiratory flow (MMEF). Statistical analysis included Wilcoxon's signed rank test and Spearman's correlation coefficients.

Results Baseline median (range) age and FEV_1 were 11.9 (5-17) years, and 91 (39-130)%-predicted. Median (range) TA_{CT1} and TA_{CT2} was respectively 9.5 (2-33)% and 9.0 (0-25)% ($p=0.49$). Median (range) TA_{stable} , $TA_{disappeared}$ and TA_{new} was respectively 3.0 (0-12)%, 5.0 (1-22)% and 7.0 (0-20)%. Visual assessment suggested that predominantly TA_{stable} was accurately assessed. TA_{CT} correlated significantly with $(TLC_{bb} - TLC_{he})/TLC_{bb}$ ($r_s=0.58$, $p=0.005$), FEF_{75} ($r_s=-0.53$, $p=0.003$) and MMEF ($r_s=-0.62$, $p=0.001$)

Conclusions TA_{CT} was not progressive over 2 years, and has a substantial stable component. TA_{CT} correlated significantly with TA_{PFT} .

Introduction

In cystic fibrosis (CF), the majority of the disease-related morbidity and mortality is determined by the severity of lung disease. CF lung disease starts early in life, evidenced by chest computed tomography (CT) studies showing structural lung abnormalities in infants (1-4). Of these early abnormalities, trapped air (TA), reflecting small airways disease, is considered an important finding (5). At age 3 months, TA is present in nearly two thirds of children diagnosed by newborn screening even in the absence of symptoms (6). In addition, TA occupies a substantial lung volume in most patients with end stage CF lung disease (7). Traditionally, TA was measured by pulmonary function tests (TA_{PFT}). More recently, the volume and distribution of TA can be visualized using expiratory chest CT (TA_{CT}). To our knowledge, there is no gold standard to determine TA, and how TA_{CT} relates to TA_{PFT} is not clear. A limited number of cross-sectional studies have been performed, and showed a correlation between TA_{PFT} and TA_{CT} (8-9). In addition, little is known about the change of TA over time. Studies using TA_{PFT} showed either progression (10), no change (11-12), or improvement over time (13). Two studies using semi-quantitative scoring to estimate TA_{CT} showed progression over approximately 2 years' time (14-15). The advantage of TA_{CT} is that TA severity as well as distribution can be determined. This enables the detection of even small, localized areas of TA. More recently, (semi-)automated systems have been developed to quantify and visualize the distribution of TA_{CT} (8-9). These techniques are fast, objective, and provide a more continuous measure of extent of TA. To our knowledge, these systems have never been used to study the natural course of TA in an unselected population. Although some CT studies suggest that TA_{CT} is progressive, it is unknown whether TA_{CT} reflects irreversible and/or reversible small airway changes. To investigate this, we have developed new software that combines quantitative analysis of TA_{CT} with deformable image registration to assess local changes in TA_{CT} in a longitudinal study. Using this software, we performed a pilot study with the aims to assess: 1) the change in TA_{PFT} and TA_{CT} volume over time; 2) changes in distribution of TA_{CT} over time; and 3) the relationship between TA_{CT} and TA_{PFT} and spirometric indicators of small airways disease.

Methods

Study population

In this retrospective study, we selected 30 consecutive children with CF, monitored at a single tertiary CF clinic, who could contribute two volumetric expiratory chest CT scans and PFTs between December 2006 and November 2009. All CTs and PFTs were made as part of routine care during annual check-up when clinically stable. Children on intravenous antibiotics were considered unstable and were excluded. The institutional review board approved the study protocol, informed consent from all subjects allowing use of anonymized data was obtained.

CT scanning protocol

Each child contributed two expiratory scans (CT₁ and CT₂) over two years. Scanning was performed from lung apex to base using a 6-slice CT scanner (Somatom Emotion, Siemens Medical Solutions, Erlangen, Germany) with the patient in supine position. Baseline CT₁ was made between December 2006 and October 2007 using voluntary breath holding. Patients were instructed to maximally exhale and hold their breath during scanning. Follow up CT₂ was made between January 2009 and November 2009 using spirometer control. This was done as follows: prior to scanning patients practiced the breathing maneuvers in supine position, supervised by a lung function technician. Patients were trained with their arms raised above their shoulders using nose clip and spirometer. For the expiratory scan, patients were asked to inhale maximally starting at tidal volume level followed by a maximum slow expiratory vital capacity manoeuvre and to hold their breath at the end of the expiration. The children performed the same breathing manoeuvres during CT scanning supervised by the same lung function technician. The scanning protocol for both CTs was volumetric (ultra low dose) using the following settings: rotation time 0.6-sec, tube voltage 80 kV (weight < 35 kg) or 110 kV (weight ≥ 35 kg), pitch 1.5, 6x2 mm collimation, kernel B60s, and a fixed tube current of 25 mA, i.e. an effective tube current-time product of 10 mAs. Scans were reconstructed with a 2.5 mm slice thickness, and 1.2 mm increment.

Image analysis

TA_{CT} volume and total lung volume were computed using in-house developed software. First, the lungs were segmented automatically(16). Second, a median filter (size 3x3x3) was applied to reduce noise. TA volume was defined as the volume of lung tissue within the segmented lung with an intensity value between thresholds of -975 and -850 HU (8, 17-18). Third, a single experienced observer visually checked the TA thresholds. Per scan, the observer evaluated whether the thresholds covered most TA areas appropriately. When needed, the upper threshold was adjusted in steps of 25 HU. Hence, the lower TA threshold in this study was set at a fixed value of -975, while the upper threshold varied between -900 and -675 HU. To evaluate the course of TA_{CT} over time, we compared the total TA volume

(expressed as a percentage of total lung volume) between CT_1 (TA_{CT1}) and CT_2 (TA_{CT2}). To investigate changes in the distribution of TA over time, we used deformable image registration to match CT_1 and CT_2 of the same subject. (19) We first estimated TA regions in both scans as described above. Then, the non-rigid transformation that optimally matched CT_2 to CT_1 was computed and applied to deform CT_2 to CT_1 . The same transformation was applied to the TA regions of CT_2 . Subsequently, the following parameters were computed, expressed as percentage of overlapping lung volume: 1) stable TA volume (TA_{stable}), 2) newly formed TA volume (TA_{new}), and 3) disappeared TA volume ($TA_{disappeared}$).

All images were visually checked to confirm successful lung segmentation and registration, and correct annotation of the changes in TA. This was done by comparing CT_1 and CT_2 displayed next to each other. On CT_1 , an overlay image could be switched on and off, showing the volume TA_{new} , $TA_{disappeared}$, and TA_{stable} in different colors. First, we visually examined whether TA appeared to be stable, new or disappeared. Second, we switched the overlay on and assessed whether the annotated areas matched our visual interpretation. Without further quantification, we documented for each scan whether large areas of TA were correctly indicated, and/or whether there was noise present.

Pulmonary function tests

Lung volumes were obtained using a Masterlab Body Plethysmograph (Erich Jaeger AG, Würzburg, Germany) using the panting technique following ERS guidelines (20). Spirometry was done with a Jaeger diagnostic system (Erich Jaeger AG, Würzburg, Germany) following ERS guidelines. All reference values were according to Zapletal (21). Only measurements obtained within 3 months of CT_1 (TA_{PFT1}) or CT_2 (TA_{PFT2}) were included for analysis. The following parameters were used as lung function parameters of TA; 1) residual volume (RV) to total lung capacity (TLC) ratio (RV/TLC); 2) difference in TLC measured by body plethysmography (TLC_{bb}) and helium dilution (TLC_{he}) expressed as percentage of TLC_{bb} ($(TLC_{bb} - TLC_{he})/TLC_{bb}$). In addition, the following parameters of small airways disease were used; forced expiratory flow at 75% of vital capacity (FEF_{75}) and maximum mid-expiratory flow (MMEF). We evaluated the course of TA_{PFT} over time by comparing TA_{PFT1} with TA_{PFT2} . Unfortunately, this could not be done for TLC_{bb} ($(TLC_{bb} - TLC_{he})/TLC_{bb}$), as no reliable helium dilution measurements were available for TA_{PFT2} due to a defect in the equipment's hardware. We investigated the relationship between TA_{CT} and TA_{PFT} by correlating TA_{CT} with RV/TLC ratio, $(TLC_{bb} - TLC_{he})/TLC_{bb}$, FEF_{75} , and MMEF.

Statistical analysis

The Wilcoxon signed rank test was used to test for differences between respectively TA_{CT1} and TA_{CT2} , TA_{PFT1} and TA_{PFT2} , and the change in TA (TA_{stable} , TA_{new} , $TA_{disappeared}$). Spearman's correlation coefficient (r_s) was used to evaluate the correlation

Part 3 Further validating CT as surrogate endpoint in CF

between TA_{CT} and TA_{PFT} . SPSS 15.0 software for Windows was used for all analyses, p-values of < 0.05 were considered significant.

Results

Patient characteristics

The study cohort consisted of 30 patients (14 boys, 16 girls). CT_1 and PFT_1 (spirometry and lung volume measurements) were performed on the same day for 28 patients. For 1 patient, PFT_1 was done 1 day prior to CT_1 , and for another patient PFT_1 was performed more than 3 months prior to CT_1 , and therefore excluded. In addition, 7 patients were too young for body box measurements. Thus, spirometry for CT_1 was available for analysis in 29 patients, and RV/TLC ratio and $(TLC_{bb} - TLC_{he})/TLC_{bb}$ was available for 22 patients.

CT_2 and PFT_2 (spirometry and lung volume measurements) were performed on the same day for 25 patients, for 5 patients, the median (range) time between CT_2

Table 1. Patient characteristics of the study cohort at baseline and follow up CT (n=30). Data shown are median (range), n indicates the number of observations on which the estimates are based.

Characteristic	Baseline CT1	n	Follow up CT2	n	p-value
Age (years)	11.9 (5-17)	30	13.9 (7-19)	30	Not tested
Length (meters)	1.48 (1.04-1.78)	30	1.58 (1.17-1.79)	30	<0.001
BMI (kilogram/meter ²)	17 (14-24)	30	18 (9-23)	30	0.01
FVC (% predicted)	92 (57-121)	29	93 (51-122)	30	0.46
FEV1 (% predicted)	91 (39-130)	29	88 (26-118)	30	0.22
FEF75 (% predicted)	58 (6-136)	29	47 (8-105)	30	0.01
MMEF (% predicted)	71 (12-149)	29	63 (5-110)	30	0.09
RV/TLC ratio (%)	28 (17-51)	22	30 (18-68)	27	0.99
$TLC_{bb} - TLC_{he}/TLC_{bb}$ (%)	9 (0-27)	22	*	*	*
CT trapped air (% of lung volume)	9.5 (2-33)	28	9.0 (0-25)	28	0.49

List of abbreviations

FVC: Forced vital capacity

FEV_1 : Forced expiratory volume in 1 second

FEF_{75} : Forced expiratory flow at 75% of vital capacity

MMEF: Maximum mid-expiratory flow

RV/TLC: Ratio of residual volume divided by the total lung capacity

$TLC_{bb} - TLC_{he} / TLC_{bb}$: Difference in total lung capacity measured by body plethysmography and helium dilution

* No values could be generated for CT_2 , due to unreliable helium dilution measurements.

and PFT_2 was 36 (-70 to 63) days. Two lung volume measurements were performed more than 3 months after CT_2 , and therefore excluded, and 1 patient could not be tested due to methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Thus, spirometry for CT_2 was available for analysis in 30 patients, and RV/TLC ratio was available for 27 patients.

For TA_{CT} measurements, 2 scans were excluded due to difficulties in the image analysis for the following reasons: failure to initialize lung segmentation due to severe malacia (n=1), and poor image registration (n=1). Thus, TA_{CT} analyses were based on 28 patients. All 28 CT_1 scans were made using voluntary breath holding. For CT_2 , 22 were spirometer-controlled. The remaining 6 scans were made during voluntary breath holding for the following reasons: lack of time before CT scan to train the spirometer-controlled CT maneuvers (n=2), fear for the spirometer-controlled CT procedures (n=1), MRSA infection (n=1), and no PFT technician available during scanning (n=2). Thus, our data set consisted of 6 CT pairs made using voluntary breath holds and 22 pairs consisting of 1 voluntary breath hold CT_1 and 1 spirometer-controlled CT_2 . Patient characteristics are shown in Table 1.

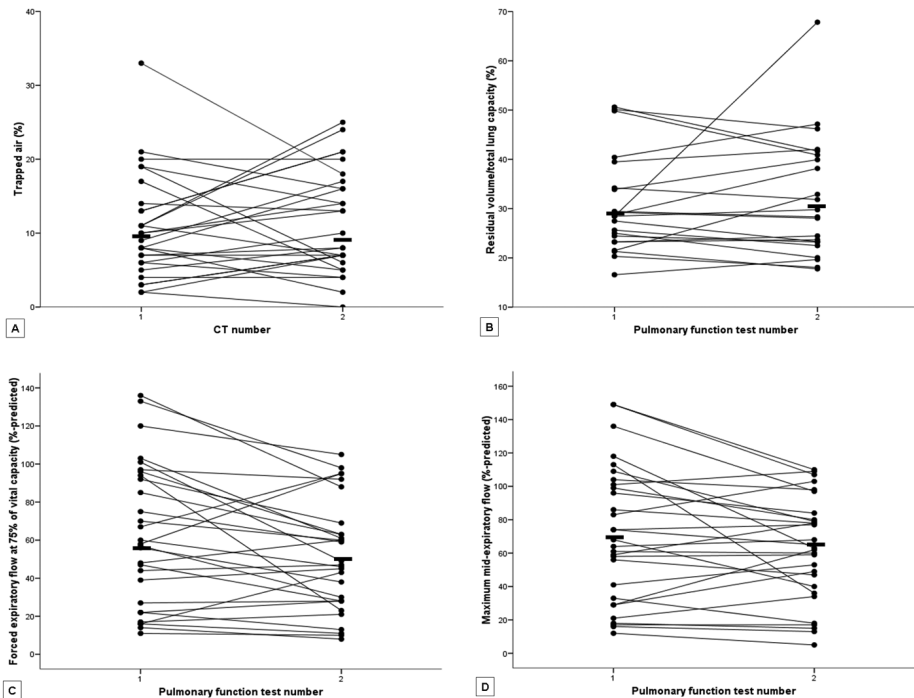


Figure 1. Plots showing the course of computed tomography estimates of relative volume of trapped air (A), pulmonary function test estimates of trapped air (B), and the spirometry measures of small airways disease forced expiratory flow at 75% of vital capacity (C) and maximum mid-expiratory flow (D) over 2 years for individual patients. Differences between the first and second measurement are only significant for plot C. Bars indicate the median values.

The course of TA over time

Longitudinal analysis of TA_{CT} showed no significant change over two years: median (range) TA_{CT_1} and TA_{CT_2} was respectively 9.5 (2-33)%, and 9.0 (0-25)% ($p=0.49$). Similarly, TA_{PFT} showed no significant change over two years: Median (range) baseline and follow up RV/TLC was respectively 28 (17-51)%, and 30 (18-68)% ($p=0.99$). Of the small airways disease parameters, only FEF_{75} was significantly decreased over time: median (range) baseline and follow up FEF_{75} was respectively 58 (6-136)%-predicted and 47 (8-105)%-predicted ($p=0.01$). The course of TA_{CT} , TA_{PFT} , FEF_{75} and MMEF over time for individual patients is shown in Figure 1.

Localized changes in distribution of TA_{CT} over time

Median (range) TA_{stable} , $TA_{disappeared}$ and TA_{new} was respectively 3.0 (0-12)%, 5.0 (1-22)% and 7.0 (0-20)%. TA_{new} and $TA_{disappeared}$ was significantly higher than TA_{stable} ($p=0.001$ and $p=0.002$). This suggests that most TA is dynamic over 2 years' time.

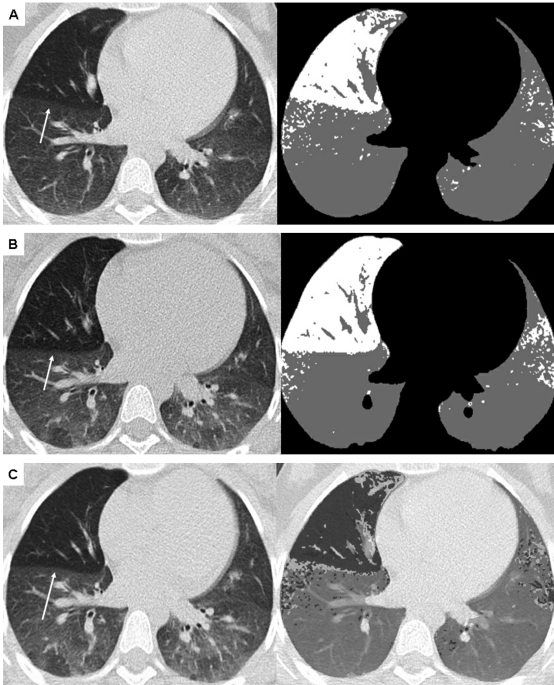


Figure 2. Images showing a CT slice of a patient of baseline CT_1 with its corresponding mask showing trapped air (TA, white) and normal lung tissue (grey) (A), follow up CT_2 with its corresponding mask (B), and CT_2 registered to CT_1 with the measured localized TA changes overlaid on CT_1 (C) all taken at the same level. The overlay shows normal lung tissue (green), and the proportion of stable TA (red), new TA (yellow), and reversed TA (blue). Colors highlight only those areas where lung segmentations in CT_1 and registered CT_2 overlap. This figure shows that the large area of stable TA in the right lung (white arrows) is correctly annotated. The relative volume for stable, new, and reversed TA in the slice shown is respectively 20.2%, 6.9%, and 2.7%. A full color version of this image can be found on page 216 in the color section

Visual assessment of the measured local changes in TA showed that large areas of TA were correctly annotated, and consisted mainly of TA_{stable} (Figure 2). TA_{new} and $TA_{disappeared}$ were frequently present as very small areas and alternated with TA_{stable} (Figure 3). Hence, the detection of TA_{new} and $TA_{disappeared}$ appeared to be noisy and may not have reflected true dynamic behavior of TA. Thus, we concluded that at least a part of TA_{CT} is stable over time, which is probably around one-third.

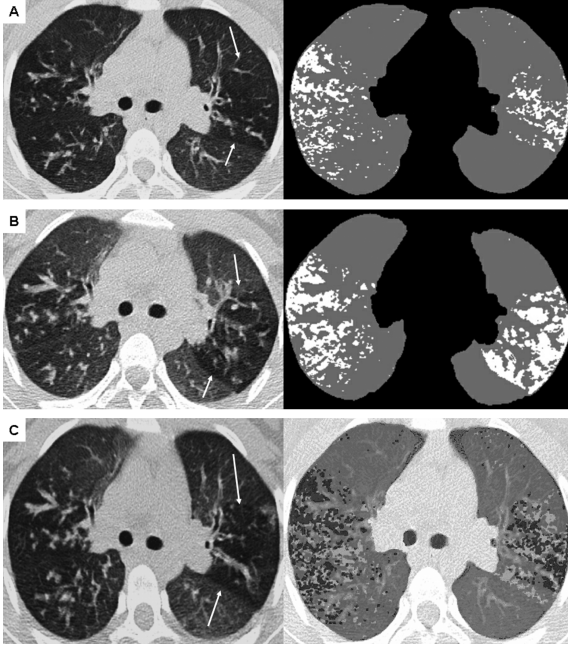


Figure 3. Images showing a CT slice of a patient of baseline CT_1 with its corresponding mask showing trapped air (TA, white) and normal lung tissue (grey) (A), follow up CT_2 with its corresponding mask (B), and CT_2 registered to CT_1 with the measured localized TA changes overlaid on CT_1 (C) all taken at the same level. The overlay shows normal lung tissue (green), and the proportion of stable TA (red), new TA (yellow), and reversed TA (blue). Colors highlight only those areas where lung segmentations in CT_1 and registered CT_2 overlap. This figure shows the noise in TA detection. Visually, the area of TA in CT_1 and CT_2 (white arrows) appear quite similar, perhaps with some slight progression. In the segmentation however, these areas were indicated as stable, new, and reversed TA with relative volumes of respectively 5.8%, 10.4%, and 4.5%. A full color version of this image can be found on page 217 in the color section

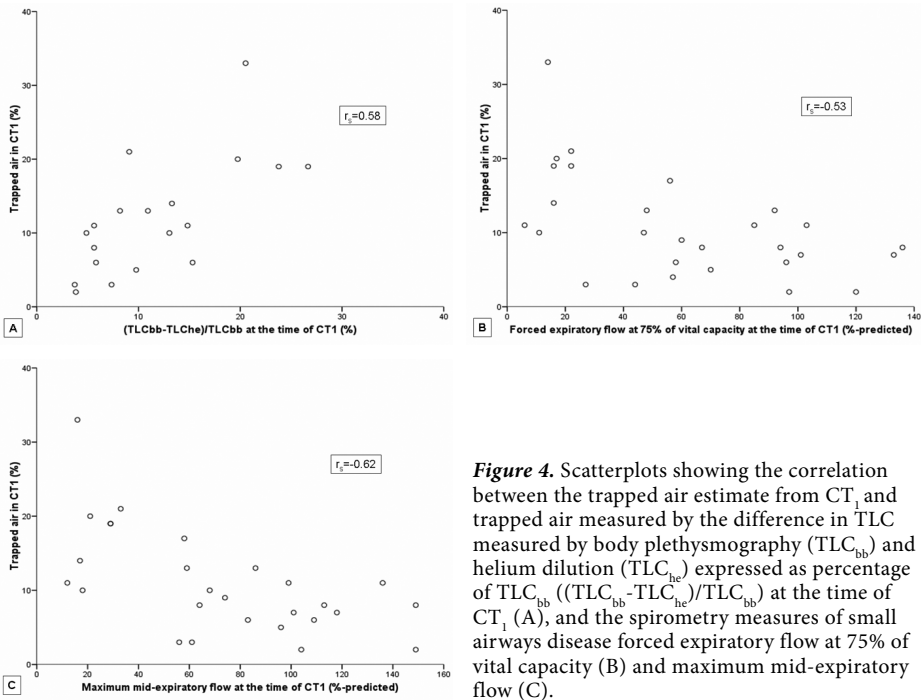


Figure 4. Scatterplots showing the correlation between the trapped air estimate from CT_1 and trapped air measured by the difference in TLC measured by body plethysmography (TLC_{bb}) and helium dilution (TLC_{hc}) expressed as percentage of TLC_{bb} ($(TLC_{bb} - TLC_{hc})/TLC_{bb}$) at the time of CT_1 (A), and the spirometry measures of small airways disease forced expiratory flow at 75% of vital capacity (B) and maximum mid-expiratory flow (C).

Relationship between TA_{CT} and TA_{PFT}

Correlations between baseline TA_{CT1} and TA_{PFT1} were only significant for TA_{CT1} and (TLC_{bb}-TLC_{he})/TLC_{bb} ($r_s=0.58$, $p=0.005$, Figure 4). Correlations between TA_{CT1} and FEF₇₅ ($r_s=-0.53$, $p=0.003$) and MMEF ($r_s=-0.62$, $p=0.001$) were all significant (Figure 4). Similar findings were found for correlations between follow up TA_{CT2} and FEF₇₅ ($r_s=-0.45$, $p=0.02$) and MMEF ($r_s=-0.43$, $p=0.02$). Correlations between TA_{CT2} and (TLC_{bb}-TLC_{he})/TLC_{bb} could not be established, as follow up (TLC_{bb}-TLC_{he})/TLC_{bb} measurements were lacking.

Discussion

To our knowledge, this is the first study to assess changes in TA_{CT} distribution over time. Our results showed that around one-third of TA_{CT} was stable over 2 years, suggesting irreversible small airway damage. Our technique is a first approach to monitor TA_{CT} distribution. Although we could not determine to what extent TA_{CT} may have been reversible, we consider it likely that not all TA_{CT} is irreversible over time. Reversibility of TA_{CT} is supported by studies showing TA_{CT} improvement after 3-months' dornase alpha treatment (22). In addition, partial reversibility of small airways disease measured by spirometry has been reported (23-27). Other treatments shown to improve small airways disease are nebulised tobramycin (28), hypertonic saline (29), and denufosal tetrasodium (30). TA is considered an important component of CF lung disease reflecting small airways disease (5). TA_{CT} is present in two-thirds of infants diagnosed by newborn screening (5-6), and forms an important component of advanced lung disease (7). TA_{CT} may be an important outcome parameter in clinical management or clinical trials aiming to reduce small airways disease. Our techniques may be of great value for monitoring treatment effects on TA volume and distribution. Whether the TA_{stable} we observed can be reversed is an important clinical question that requires further investigation.

Detection of TA_{new} and TA_{disappeared} using our technique was less robust. This may have been caused by the following issues. First, images were acquired using ultra low dose protocols. Image quality allowed TA assessment, but with a substantial degree of noise. A filter reduced the noise, but could not eliminate it. More advanced segmentation techniques, manually or automatically outlining complete regions of TA could improve results. Second, TA thresholds were visually determined. Small observer-related changes may have influenced whether TA was annotated as new, reversed or stable. Third, differences in scan exhalation level may have affected the measurements. Baseline CTs were made using voluntary breath holds, while 22/28 follow up CTs were made using spirometer-control. Voluntary breath hold scans are likely close to functional residual capacity (31), while spirometer-controlled scans are close to RV. Subgroup analysis comparing 8

voluntary breath hold pairs with 22 pairs with 1 voluntary breath hold CT₁ and 1 spirometer-controlled CT₂ revealed no differences in TA_{CT} volume or distribution over time (data not shown). Ideally, our findings should be reproduced using two spirometer-controlled CTs. Fourth, lung growth may have affected the measurements. Median age in our cohort at baseline approached 12 years, the age at which growth accelerates in healthy children. Mean total lung growth for 12-year-olds has been estimated at 300 ml (girls) and 600 ml (boys) over 2 years (32). Little is known about how lung growth affects TA_{CT}, and whether lung growth in CF patients resembles that of healthy children. We used deformable image registration to match CTs, which may have partly compensated for effects of growth (19).

Cross-sectional analysis showed significant correlations between TA_{CT} and $(TLC_{bb} - TLC_{he})/TLC_{bb}$, FEF₇₅, and MMEF. These findings support the accuracy of our TA_{CT} volume estimate to reflect small airways disease. In contrast to other studies (8-9), we found no correlation between TA_{CT} and RV/TLC. These differences could be due to differences in CT analysis or CT protocol.

Longitudinally, we found no change in total TA_{CT} volume over time. Other studies showed TA_{CT} progression over two years (14-15). These studies however, used younger (14) or older (15) CF patients, limited slice protocols, and manual scoring methods. Similarly, TA_{PFT} did not change over time, which replicates results of 2 other studies (11-12). However, TA_{PFT} progression (10), and improvements (13) over time have also been described. These differences can only be partly explained by differences in TA measure used and study duration (10). A limitation was the absence of CT₂ values of $(TLC_{bb} - TLC_{he})/TLC_{bb}$. The $(TLC_{bb} - TLC_{he})/TLC_{bb}$ has been used to estimate TA (33), but never longitudinally.

Only FEF₇₅ decreased significantly over time, a finding supported by other studies (5, 34-37). These results suggest that FEF₇₅ was the most sensitive measure to monitor small airways disease. However, sample size was small, and larger studies are needed to confirm our observation. Furthermore, our software can likely be improved in the near future, which may improve its sensitivity to track changes in TA_{CT}. In addition, CT's advantage of visualizing patterns of TA may enhance the detection of TA subtypes, which may warrant tailored treatment. The drawback of CT however, is radiation exposure. The current protocol can acquire expiratory CTs by administering approximately 0.4 milli Sievert (31). Keeping the risk-benefit ratio in mind, monitoring TA using CT is likely to be valuable and safe.

In conclusion, the importance of small airways disease in CF is well recognized. However, little is known on its pathophysiology, treatment, reversibility, and monitoring. TA detection by CT can improve our understanding of small airways disease. In this pilot study, we describe a technique to assess TA volume and distribu-

Part 3 *Further validating CT as surrogate endpoint in CF*

tion over time, which can be used to monitor TA treatment in clinical practice and clinical trials. TA_{CT} detection can be further improved using spirometer-control and improved segmentation techniques. Further longitudinal studies comparing TA_{CT} and TA_{PFT} are needed to identify the most optimal strategy to monitor small airways disease.

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Chapter 6

Bronchiectasis and pulmonary
exacerbations in children
and young adults with Cystic
Fibrosis

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Abstract

Objective Respiratory tract exacerbation rate (RTE-R) is a key clinical efficacy endpoint in cystic fibrosis (CF) trials. Chest computed tomography (CT) holds great potential as a surrogate endpoint. Evidence supporting the ability of CT scores to predict RTE-R is an important step in validating CT as a surrogate endpoint. The objective of this study was to investigate the association between CT scores and RTE-R in a cohort of pediatric CF patients.

Methods Retrospective review of data from pediatric CF patients included chest CT scans, spirometry, and two years follow up. RTE-R was defined as number of intravenous antibiotics courses per year. CT scans were scored with the Brody-II system assessing bronchiectasis, airway wall thickening, mucus, and opacities.

Results One hundred fifteen patients contributed 170 CTs. Median age and FEV₁ at first CT were 12 years (range, 5-20 years) and 90% predicted (range, 23% predicted -132% predicted). Analyzing exacerbation counts using Poisson regression models, bronchiectasis score and FEV₁ were both found to be strong independent predictors of RTE-R in the subsequent 2 years. For the bronchiectasis score categorized in quartiles, RTE-R increased by factors of 1.8 (95% CI 0.6-6.1; p=0.31), 5.5 (1.9-15.4; p=0.001), and 10.6 (3.8-29.4; p<0.001), respectively, for each quartile compared to the quartile with the best, i.e. lowest, scores. Similarly, time to first RTE was significantly associated with quartiles of both bronchiectasis score and FEV₁.

Conclusions The CT scan bronchiectasis score is strongly associated with RTE-R in pediatric patients with CF, providing an important piece of evidence in the validation of CT scans as an endpoint for CF clinical trials.

Introduction

In cystic fibrosis (CF), many endpoints for clinical trials have been evaluated, including respiratory tract exacerbation rate (RTE-R), spirometry, bronchoalveolar lavage markers and imaging.(1-2) Of these endpoints, only RTE-R is considered a clinical outcome parameter, whereas the others are surrogate endpoints. Unfortunately, RTE-R lacks a standardized definition, and frequently requires a relatively large sample size to detect a relevant treatment effect.(3) Therefore, surrogate endpoints have been used, of which forced expiratory volume in one second (FEV_1) is the most common.(4) However, FEV_1 has become relatively insensitive for monitoring the progression of CF lung disease because average lung function is now in the normal range until adolescence, and average annual FEV_1 decline is currently less than 1% per year.(5) FEV_1 also is insensitive to important localized structural damage such as bronchiectasis.(6) Thus, there is a need for more sensitive surrogate endpoints with the ability to detect disease onset and progression at an early stage.

Computed tomography (CT) has great potential for use as a surrogate endpoint in CF lung disease because it is the gold standard for the detection of bronchiectasis, the defining structural abnormality in CF.(7) CT scanning has been shown to be more sensitive than FEV_1 in detecting early CF lung disease and in monitoring disease progression.(8) In addition, CT parameters such as mucus plugging and centrilobular nodules have been shown to respond to treatment.(9) Importantly, the use of CT-related parameters instead of FEV_1 could potentially reduce the sample size in intervention studies,(10) significantly enhancing the feasibility of clinical studies in CF.

An important step in the validation process of CT as a clinical trial endpoint is establishing its association with clinical outcomes, such as RTE-R.(3) To date, the association between CT scores and RTE-R has only been investigated in one small selected cohort. Among 61 CF patients aged 6-11 years with well preserved lung function participating in the Pulmozyme Early Intervention Trial, Brody et al (11) found a statistically significant but relatively poor correlation between CT scores and RTE-R. Whether CT scan-related parameters correlate with RTE-R in an unselected CF population has never been investigated. Therefore, as one step in validating CT scores as a clinical trial endpoint, our primary aim was to investigate the predictive value of CT scan scores for RTE-R in the ensuing 2 years. Because lung function is the most widespread surrogate endpoint in CF clinical trials, a secondary objective was to evaluate the predictive value of CT scores while taking account of spirometric measurements regarding RTE-R in the ensuing 2 years.

Methods

Study population

This institutional review board-approved, retrospective, single-center study used clinical data from patients with CF followed at the Sophia Children's Hospital Cystic Fibrosis Clinic in Rotterdam, The Netherlands. Inclusion criteria were: (1) confirmed CF diagnosis, (2) one or more routine biannual chest CT scans performed between March 2002 and March 2006 while clinically stable (CT scans performed for acute respiratory deterioration were not included in the current analysis), (3) at least one routine spirometry while clinically stable within 4 months of each CT, (4) age between 5 and 20 years at the first CT scan, (5) ³2 years of follow up after each CT, and (6) informed consent. Exclusion criteria were: (1) CT scans performed under general anaesthesia (due to risk of anaesthesia-induced atelectasis), (2) non CF-related lung abnormalities, and (3) co-morbidity potentially affecting RTE-R (severe tracheomalacia [n=1] and IgM deficiency [n=1]).

Because there is no universal consensus on the definition of a respiratory tract exacerbation (RTE) (12), we selected a conservative approach and defined RTEs as episodes of treatment with intravenous (IV) antibiotics for pulmonary indications, as used in other studies.(11, 13-14) For each subject, annual RTE-R was determined by detailed chart review. For one subject with severe lung disease requiring continuous IV treatment of > 1 year, the number of exacerbations was arbitrarily set at 10, a number larger than the highest observed count in the cohort. *Pseudomonas aeruginosa* culture positivity was defined as the presence of one or more positive respiratory cultures at any point in time before each CT scan (*Pseudomonas* positivity ever).

CT scanning protocol

Scans were performed on two scanners (Prospeed SX; GE Medical Systems; Waukesha, Wisconsin, and Somatom Emotion; Siemens Medical Solutions; Erlangen, Germany) in the supine position from apex to base. Only inspiratory scans were analyzed because protocols before September 2003 did not include expiratory acquisitions. All scans were performed during voluntary breath holds at end inspiration. CT scans performed from March 2002 to July 2004 were high-resolution scans and obtained at 80- to 120-kVp and 100- to 130 mA fixed tube current, 1.0 mm slice thickness at 5- to 10-mm intervals, 0.8-1.0-s rotation time, and high spatial frequency algorithm. Average radiation dose for these protocols was 0.9 mSv (based on a mean gap of 7.5 mm, and calculated using the impact dosimetry calculator (15), and multiplied with pediatric normalized values (16). From August 2004 to March 2006, volumetric CT scans were obtained at 80- to 110-kVp and 20-mAs reference tube current, with 2-mm collimation, 3-mm slice thickness, 0.6-s rotation time, pitch 1.5 and high spatial frequency algorithm. Average radiation dose for this protocol was approximately 1 mSv (inspiratory scan, 0.69 mSv, plus expiratory scan, 0.35 mSv).

CT scoring and spirometry

Scans were scored with the Brody-II scoring system, evaluating bronchiectasis, airway wall thickening, mucus plugging, and opacities (17). Trapped air was excluded from the total score. Hence, the maximal possible total score (207 points) was reduced by 27 points for trapped air, changing the maximum score to 180 points. Scores were expressed as percentages of maximal scores on a zero-to-100 scale. All scans were de-identified, randomized and scored by one experienced observer (K.G.) (18). For within-observer variability, this observer re-scored 25 random scans after one month. For between-observer variability, a second blinded observer (M.L.) scored 25 random scans. Both observers were blinded to lung function results and clinical status. The observers were trained using a scoring manual including reference images, and established good interobserver and intraobserver agreement prior to scoring CT scans for the current study. Spirometry was performed using the Jaeger diagnostic system (Jaeger AG, Hoehberg, Germany). FEV₁, forced vital capacity (FVC), and forced expiratory flow at 75% of vital capacity (FEF75) were analysed. All reference values were according to Zapletal et al.(19)

Statistical analysis

Descriptive statistics were used to characterize the patients at the time of their first CT scan. For patients with two CTs, the paired *t* test, and Wilcoxon signed rank

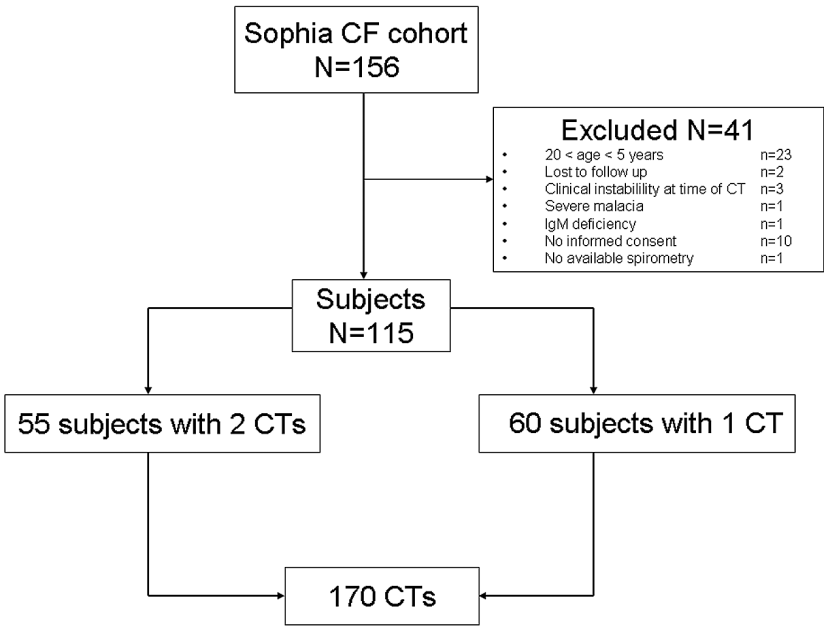


Figure 1. Flow chart of the study cohort. CF=cystic fibrosis

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test were used to compare spirometry, FEV_1 , and CT scan scores, respectively, at the time of the first versus the second CT scan.

To evaluate the predictive value of CT score and spirometry on annual RTE-R in the subsequent two years, we used univariate and multivariable Poisson regression with generalized estimating equations to account for the correlation between repeated measures within an individual using SAS, PROC GENMOD (SAS Institute; Cary, North Carolina). To account for the effect of the temporal change in CT scanning protocols, CT scans performed between March 2002 and July 2004 were coded CT1, and CT scans between August 2004 and March 2006 were coded CT2, with $\log e$ (observation time) per period as an offset. CT scores and spirometric measurements were grouped into quartiles based on their observed distributions in the study population, with the lowest CT score or highest FEV_1 quartile as the reference category. This grouping into quartiles was chosen, because there are no “natural” cut off levels for the CT scores. The same grouping into quartiles was used for spirometry data. Kaplan Meier curves were used to assess the time from each CT to the first subsequent RTE, and the log rank test was used to evaluate differences between the quartiles.

Table 1. Baseline characteristics of the study cohort.

Characteristic	Study cohort
N	115
Males	55 (48%)
Age (years)	11.9 (5-20)
FEV_1 (% pred)‡	91 (23-132)
FVC (% pred)*	94 (41-122)
FEF_{75} (% pred) [#]	50 (6-151)
Positive <i>Pseudomonas aeruginosa</i> culture ever**	76 (67%)
Brody-II total score (%)	7.2 (0-36)
Bronchiectasis score (%)	6.9 (0-39)
Airway wall thickening score (%)	5.6 (0-39)
Mucus plugging score	8.3 (0-44)
Opacity score (%)	5.6 (0-13)

Data are presented No. (%) or as median (range), unless otherwise indicated.

‡Forced expiratory volume in 1 second

*Forced vital capacity

[#]Forced expiratory flow at 75% of vital capacity

** Includes all *P. aeruginosa* cultures ever before the time of the first CT scan.

Intraclass correlation coefficients (ICCs) were used to evaluate between- and within-observer agreement for CT scan scores. ICC values between 0.4 and 0.6, 0.6 and 0.8 or ≥ 0.80 generally are considered to indicate moderate, good and very good agreement, respectively. SPSS, version 15.0 (SPSS Inc; Chicago, Illinois) and SAS, version 9.2, statistical software were used for analyses. $P < 0.05$ (two-sided) was considered statistically significant.

Results

Study population

We identified 156 patients who had at least one chest CT during the study period. From this cohort, 41 patients were excluded for reasons outlined in Figure 1. Thus, 115 patients were included in the current analyses, with 55 contributing two scans and 60 contributing one scan, for a total of 170 scans, Total person-years of follow up was 335. The mean \pm SD follow-up period was 23.6 ± 2.2 months after each CT. Spirometry was performed on the same day as CT scanning for 149 of 170 observations. For the 21 remaining observations, the median time between CT and spirometry was 0 days (range, -42 to 125 days). Baseline subject characteristics are shown in Table 1.

Table 2. Spirometry, Pseudomonas culture status and CT scan scores at the time of the first and second CT scans among subjects contributing two CT scans (n=55).

Characteristic	First CT	Second CT	Difference (95% CI)	p-value
FEV ₁ (% pred)	93 \pm 13	88 \pm 16	-5 (-8 to -2)	0.005
FVC (% pred)	94 \pm 12	92 \pm 14	-2 (-4 to 1)	0.47
FEF75 (% pred)	58 \pm 23	49 \pm 27	-9 (-15 to -3)	0.001
Positive P. aeruginosa culture ever*	35 (64%)	42 (76%)	12 (3 to 21) %	...
Brody-II total score (%)	6.7 (0-35)	9.4 (0-35)	1.7 (1.1 to 3.5)	<0.001
Bronchiectasis score (%)	5.6 (0-39)	8.3 (0-46)	1.4 (0.9 to 4.2)	0.003
Airway wall thickening score (%)	5.6 (0-39)	5.6 (0-35)	0 (-0.7 to 2.9)	0.22
Mucus plugging score	5.6 (0-31)	8.3 (0-39)	0 (0 to 3.0)	0.005
Opacity score (%)	5.6 (0-13)	7.4 (0-15)	1.9 (0-1.9)	<0.001

Data are presented as mean \pm SD, median (range) or No. (%), unless otherwise indicated. See Table 1 legend for expansion of abbreviations.

* includes all P. aeruginosa cultures ever before the time of CT. The p value is not calculated, as this percentage can only increase.

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Among the 55 subjects contributing two CT scans, a significant decrease in lung function (except for FVC), and increase in CT scores (except for airway wall thickening subscore) was observed between the first and second CT scans (Table 2). Mean \pm SD time between the first and second CT was 1.9 ± 0.31 years.

Among the 115 subjects, 51 (44%) experienced a total of 148 RTEs during follow up. The mean annual RTE-R in the two years following each CT scan by quartile of CT scan score and spirometric measurements, derived from univariate Poisson modeling, is shown in Table 3. (Definitions of the parameters for all quartiles are presented in Table 4.) RTE-R increased significantly with both worsening lung function and CT scan score.

In separate multivariable models for spirometry and CT scan scores, the strongest spirometric predictor for annual RTE-R was FEV₁, with FVC and FEF₇₅ not adding significantly to the model. For CT scan scores, the bronchiectasis score was

Table 3. Annual number of RTEs during the two years following spirometry and CT scans by observed quartile of the parameter in the study population.^a

Spirometry	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-value
FEV ₁	0.12 (0.06-0.24)	0.24 (0.12-0.50)	0.25 (0.12-0.52)	1.18 (0.87-1.59)	<0.001
FVC	0.20 (0.12-0.35)	0.15 (0.06-0.35)	0.34 (0.18-0.63)	1.06 (0.75-1.49)	<0.0001
FEF75	0.15 (0.05-0.40)	0.17 (0.09-0.30)	0.27 (0.15-0.49)	1.03 (0.75-1.41)	<0.0001
CT scan scores					
Bronchiectasis	0.06 (0.02-0.14)	0.11 (0.06-0.21)	0.52 (0.36-0.76)	1.18 (0.84-1.67)	<0.0001
Airway wall thickening	0.12 (0.06-0.23)	0.19 (0.11-0.32)	0.48 (0.27-0.88)	1.08 (0.76-1.52)	<0.0001
Mucus plugging	0.09 (0.04-0.20)	0.33 (0.16-0.71)	0.43 (0.27-0.69)	1.04 (0.72-1.50)	<0.0001
Opacity	0.10 (0.05-0.23)	0.15 (0.07-0.30)	0.54 (0.37-0.79)	1.42 (0.97-2.11)	<0.0001
Brody-II total	0.07 (0.03-0.16)	0.15 (0.08-0.27)	0.45 (0.26-0.80)	1.13 (0.82-1.54)	<0.0001

Data are presented as mean (95% CI). P values are for the overall difference among quartiles from univariate Poisson regression models. RTE=respiratory tract exacerbation. See Table 1 legend for expansion of abbreviation.

^aSee Table 4 for definitions of quartiles for all parameters

the strongest predictor, with no additional significant effect of the other CT scan scores, including the total score (data not shown).

Table 4. Definitions of parameters for all quartiles.

parameter	quartile 1	quartile 2	quartile 3	quartile 4
FEV ₁	> 100	90-100	79-89	≤78
FVC	> 101	95-101	85-94	≤84
FEF ₇₅	> 70	50-70	31-49	≤30
Bronchiectasis score	≤ 1	2-6	7-15	≥16
Airway wall thickening score	≤ 1	2-5.6	5.7-11	≥12
Mucus plugging score	≤ 1	2-8.3	8.4-16	≥17
Opacity score	≤ 3.7	3.8-5.6	5.7-9.2	≥9.3
Brody-II total score	≤ 2.9	3-8	9-14	≥15

Spirometry is presented as % predicted, and CT scan scores are presented as % of maximal scores. See Table 1 legend for expansion of abbreviations.

Table 5 shows the results of the multivariable Poisson regression model, including FEV₁ and bronchiectasis score; age was not included because it did not add significantly to the model (p=0.83). These results show that both FEV₁ and the bronchiectasis score have significant, independent predictive values for RTE-R in the ensuing 2 years.

Table 5. Exacerbation rate ratios (RR) according to FEV₁ and CT scan bronchiectasis score.

Parameter	RR	95% CI	p-value
FEV ₁ (%-predicted)			
> 100	1*	-	-
90-100	1.9	0.7-5.0	0.21
79-89	1.8	0.6-5.1	0.27
≤78	3.9	1.7-8.9	0.001
Bronchiectasis score (%)			
≤ 1	1*	-	-
2-6	1.8	0.6-6.1	0.31
7-15	5.5	1.9-15.4	0.001
≥16	10.6	3.8-29.4	<0.001

*From the multivariable Poisson regression model including FEV₁ and bronchiectasis score quartiles, adjusted for CT number. Both parameters were divided into quartiles as detailed in Table 3.

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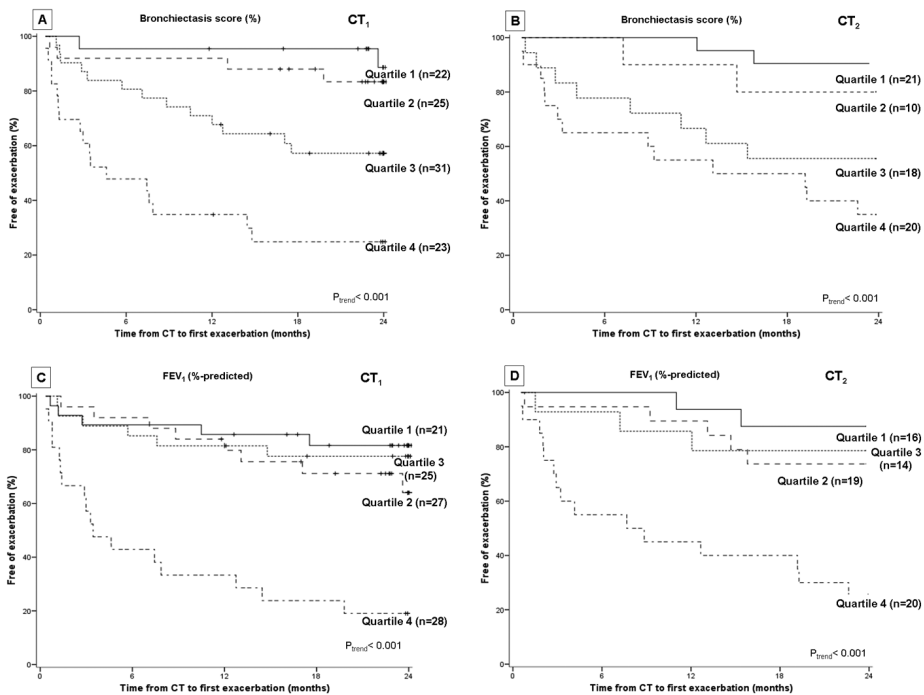


Figure 2. Kaplan-Meier plots for time to first respiratory tract exacerbation after CT scan. Individuals are grouped by quartile of bronchiectasis score (plots A and B) or FEV₁ (plot C and D). Quartiles for FEV₁ (% predicted) are as follows: quartile 1 = > 100, 2 = 90-100, 3 = 79-89, 4 = £78, and for bronchiectasis score (%): quartile 1 = £ 1, 2 = 2-6, 3 = 7-15, 4 = ³16. P-values are derived from log-rank test for trend.

Kaplan Meier plots of time to first RTE according to bronchiectasis score and FEV₁ quartile are shown in Figure 2. Time to first RTE was significantly associated with quartiles of both bronchiectasis score and FEV₁.

Reproducibility was good for all CT scan scores. ICCs for within-observer agreement were all above 0.95, while between-observer agreement ranged from 0.61 (mucus plugging) to 0.86 (total score).

Discussion

The U.S. Food and Drug Administration defines a surrogate endpoint as “a laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient feels, functions, or survives.”(20) Surrogate endpoints, such as CT scan scores, generally are used as a substitute for true clinical efficacy measures, such as RTEs, when the

clinical benefit may not be detectable in trials of reasonable cost, duration, or size. (3) Food and Drug Administration regulations state that a surrogate endpoint is considered to be “reasonably likely to predict clinical benefit and, therefore, useable for drug approval if there is evidence based on epidemiologic, therapeutic, pathophysiologic, or other data supporting the association of the surrogate with the clinical benefit.(21)

In the present study, we demonstrate the clear association between CT scan scores, particularly the bronchiectasis score, and RTEs in a cohort of patients with CF. This step is important in the process of validating CT scanning as a surrogate endpoint for CF clinical trials. The association between bronchiectasis and RTEs has been observed previously in a small cohort of young patients with CF with mild disease enrolled in a clinical trial (11), in non-CF bronchiectasis patients (22), and in patients with chronic obstructive pulmonary disease.(23) In CF, RTE-R is an important clinical endpoint for intervention studies. RTE-R has been shown to increase with age and more severe lung function impairment.(24) Furthermore, RTE rates are clearly associated with survival.(25) Unfortunately, RTE-R is a relatively insensitive endpoint, especially in patients with well preserved lung function, and requires a large sample size when used in clinical studies.(3) In addition, there is no consensus regarding the definition of an RTE.(12) Currently, FEV₁ is still the most widely used surrogate endpoint; however, its use as surrogate endpoint has substantial limitations. First, the annual change in FEV₁ has become so small that intervention studies using rate of decline in FEV₁ as the primary endpoint would require large sample sizes.(10) In addition, FEV₁ has poor sensitivity to detect early structural airway damage.(8) It has been estimated that the use of bronchiectasis scores as a surrogate endpoint would require a smaller sample size (10) and would increase the feasibility of clinical trials in CF. Other arguments that favor the use of the bronchiectasis score as a surrogate endpoint are that bronchiectasis is progressive,(8) detectable early in the disease process,(26) an important component of end stage CF lung disease (27), and associated with impaired quality of life.(28) An important next step in validating CT scanning as a surrogate endpoint will be to demonstrate that the effect of a therapy on the CT score predicts the drug’s effect on a clinical endpoint, such as RTE.(3).

To be able to use CT parameters such as the bronchiectasis score as endpoints in multi-center trials, CT scanning protocols and image analysis will need to be standardized to avoid bias related to differences in image resolution and density distribution, and to improve the sensitivity and reproducibility of the scores. In addition, to use CT scanning in clinical studies, it is of utmost importance that the radiation dose be minimized. Scan protocols requiring low doses of radiation without losing relevant information have been developed.(29) The volumetric CT scan protocol used in this study, which we currently still use in clinical practice, can acquire volumetric inspiratory and expiratory scans with a mean total effec-

tive dose of approximately 1 mSv (depending on tube voltage and patient size). (18) This is comparable to one-third of the annual US background radiation dose. (30) These doses can likely be further reduced in the near future. (18) Keeping the risk-benefit ratio of clinical trials in mind, the bronchiectasis score should be considered as an endpoint in studies aiming to slow the progression of CF lung disease. Although the use of magnetic resonance imaging (MRI) in patients with CF as an alternative to CT seems promising, (31) the spatial resolution of MRI, and therefore its use in the assessment of bronchiectasis, is currently still inferior to that of CT. (32) For spirometry, methods such as lung clearance index may prove to be good surrogate markers for early disease in the future (2, 33-34). However, this needs to be further investigated.

The present study has several limitations. First, it was retrospective; therefore, we had to select a robust and conservative definition for RTEs by defining it as the need for IV antibiotic treatment for pulmonary deterioration and increased symptoms. This data was easily extracted and validated using our electronic patient record. Unfortunately, there is no accepted consensus for RTEs in CF. Our definition has been used in other studies. (11, 13-14) Using this definition, RTEs were unlikely to be missed. However, any lack of ascertainment of RTEs would be unlikely to alter the association between CT scores and RTE-R. Secondly, this study was performed in a single center, potentially affecting the generalizability of the results. Although we included patients aged up to 20 years, our cohort consisted of relatively few adult patients with severe lung disease (n=8 with FEV₁ 35-49%-predicted, severe as defined by American Thoracic Society/European Respiratory Society criteria). (35) Hence, our model may not be adequate for the adult population with more advanced lung disease. Similarly, the model's fit on an infant CF population could not be studied, as children aged < 5 years were not included in this study. Whether bronchiectasis on CT scans is predictive for RTE in children aged < six has to be further investigated, since the nature of exacerbations and the frequency may be different in these children relative to the population included in our study. Third, the importance of trapped air on CT images could not be established, since 40/170 scans did not contain expiratory images. In general, the importance of trapped air is less well established than that of bronchiectasis. Trapped air is present early in the disease process (26), and in end-stage lung disease. (27) However, its reversibility and impact on quality of life have not been established. Future studies are needed to determine the importance of trapped air as a possible surrogate endpoint in CF. Fourth, the CTs in this study were performed using two different scanners and, thus, potentially could have introduced some bias related to differences in resolution and density distribution. However, we consider it unlikely that the differences in scanning techniques were substantial enough to cause significant differences in CT scores. In addition, we used a manual scoring system that generally is thought to be less sensitive to differences between CT scans and protocols (10, 36). Despite these limitations, the results showed that the

bronchiectasis score has significant additional predictive value for RTE-R beyond that provided by FEV₁ in children and adolescents with mild to moderate CF lung disease, supporting further validation in a prospective study using the bronchiectasis score as a clinically relevant surrogate endpoint in clinical trials designed for this patient population.

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Chapter 7

Chest Computed Tomography scores are predictive of survival in CF patients awaiting lung transplantation

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Abstract

Background Up to a third of cystic fibrosis (CF) patients awaiting lung transplantation (LTX) die while waiting. Inclusion of computed tomography (CT) scores may improve survival prediction models such as the lung allocation score (LAS). This study investigated the association between CT and survival in CF patients screened for LTX.

Methods Clinical data and chest CTs of 411 CF patients screened for LTX between 1990 and 2005 were collected from 17 centers. CTs were scored with the Severe Advanced Lung Disease (SALD) 4-category scoring system, including the components 'infection/inflammation' (INF), air trapping/hypoperfusion (AT), normal/hyperperfusion (NOR) and bulla/cysts (BUL). The volume of each component was computed using semi-automated software. Survival analysis included Kaplan-Meier curves, and Cox-regression models.

Results 366 (186 males) out of 411 patients entered the waiting list (median age 23, range 5-58 years). Subsequently, 67/366(18%) died while waiting, 263/366(72%) underwent LTX, and 36/366(10%) were awaiting LTX at the census date. INF and LAS were significantly associated with waiting list mortality in univariate analyses. The multivariate Cox model including INF and LAS grouped in tertiles and comparing tertiles 2 and 3 to tertile 1, showed waiting list mortality hazard ratios of 1.62 (95%CI 0.78-3.36, $p=0.19$), and 2.65 (1.35-5.20, $p=0.005$) for INF and 1.42 (0.63-3.24, $p=0.40$), and 2.32 (1.17-4.60, $p=0.016$) for LAS, respectively. These results indicated that INF and LAS had significant, independent predictive value for survival.

Conclusions CT score INF correlates with survival, and adds to the predictive value of LAS.

Introduction

In cystic fibrosis (CF), life expectancy has greatly improved, and median age now approaches 38 years.(1) However, most patients still develop severe advanced lung disease (SALD), which accounts for 85% of deaths in this patient population.(2) For CF patients with SALD, lung transplantation (LTX) is often the only treatment option left that may provide survival benefit and improves quality of life.(3) Currently, CF is the third leading indication for LTX in adults, and the most common indication for LTX in children aged 6 years and older.(4-5) Choosing the appropriate time for referral for LTX remains a challenge. In 1998, the American Thoracic Society (ATS) published guidelines to assist physicians in identifying potential candidates for LTX.(6) Despite these guidelines, mortality estimates for CF patients awaiting LTX as high as 37% have been reported.(7-9) This urged studies to search for better outcome measurements of survival (10-17), leading to an update of the guidelines in 2006.(18) Presently, referral for LTX assessment is advised when 2- to 3-year predicted survival is less than 50%, using the following CF-specific criteria: forced expiratory capacity in 1 second (FEV_1) below 30%-predicted or rapid respiratory deterioration in subjects with an FEV_1 greater than 30%-predicted, particularly in young females; acute pulmonary exacerbations requiring intensive care; increasing frequency of acute pulmonary exacerbations; refractory and/or recurrent pneumothorax, and recurrent hemoptysis not controlled by embolization.(18)

Another approach towards reducing waiting list mortality was the introduction of the lung allocation score (LAS) in the United States in May 2005.(8) This score balances pre- and one-year post-transplant survival using clinical parameters with subsequent proven survival effect.(8, 19)

The relationship of lung structure information and survival has never been studied. This data is readily available, although not systematically interpreted, as chest computed tomography (CT) scans are routinely incorporated in most LTX assessment protocols worldwide.(20) There is substantial evidence that CT could add highly relevant information to current survival prediction models. First, FEV_1 below 30%-predicted is an important screening criterion.(18) However, in mild to moderate CF, lung function correlates poorly with the severity of structural changes on CT.(21-23) CT is more sensitive than pulmonary function tests (PFTs) in detecting onset and progression of CF-related lung disease.(21-24) Second, severity of CF bronchiectasis on CT is predictive of acute pulmonary exacerbations (25-26), and has a negative impact on the quality of life.(27) We hypothesized that structural abnormalities on CT would predict survival prior to LTX.(28) Therefore, we conducted the current study to: 1) examine the association between CT and survival in CF patients listed for LTX, 2) investigate the prognostic value to LAS added by CT, and 3) determine the spectrum of structural abnormalities in SALD and its correlation with PFTs.

Methods

Study population

CF patients screened for LTX between January 1 1990 and December 31 2005 in 17 centers were included if clinical screening data, including a chest CT within the preceding 12 months was available. We collected demographic information, weight and height, sputum microbiology, hematology, blood chemistry, and pulmonary function data (online supplement). Data was collected by chart review by a single investigator (ML) during site visits in 16 sites and by review of the electronic medical record at one site. Selection criteria in all centers were based on the 1998 ATS guidelines.(6) One center used alternative values for FEV₁ i.e <25%-predicted (males) and <40%-predicted (females). The human subjects review boards at all centers approved the study protocol and waived written informed consent because of the observational nature of the study.

CT scanning procedures

CTs were made using a range of scanners and protocols (online supplement, Table E1). Scans were available in two formats; 208(51%) films (January 1991 to November 2005), and 203(49%) digital scans (October 1996 to December 2005). Scans on film were digitized. All scans were anonymized and copied on compact disc.

CT analysis

CT analysis was done in a single batch. Before scoring, image quality was rated (good/medium/poor), based on image resolution and movement artefacts. Scans with poor quality were excluded from scoring. Scans were scored in randomized order using the SALD scoring system.(28) In this system, all lung tissue is divided into 4 components of lung morphology. Three components indicate abnormalities: 1) infection/inflammation (INF, including bronchiectasis, airway wall thickening, mucus and consolidation), 2) air trapping/hypoperfusion (AT), and 3) bulla/cysts (BUL). The fourth component normal/hyperperfused tissue (NOR) reflects normal or hyperperfused parenchyma. We applied a novel semi-automated approach to the SALD scoring system to improve precision and reduce analysis time, by using an in-house developed software tool (MeVis Medical Solutions AG and Fraunhofer MEVIS, Bremen, Germany). Scans were annotated using a standardized procedure (online supplement). In brief; a 10x10 mm grid was projected over each slice and each grid cell was manually assigned to one of the 4 components, using different colors (online supplement, Figure E1). To speed up the manual annotation process, lung tissue was segmented using region growing with one manual seed point per lung in the color of the component predominantly present in the scan.

A random subset of CTs (online supplement) showed that scoring 1 slice every 30 mm was sufficient to compute reproducible and accurate scores, thus analyzing

6-9 slices per scan. Scoring was done by a single experienced observer (ML), who was blinded to clinical data and outcome. Within observer agreement was established by re-scoring 25 random scans after one month.

Statistical analysis

To define the spectrum of abnormalities, data from all patients assessed but not listed ($n=411$) was used. For survival analysis, only data from patients listed after screening was used ($n=366$). The Mann-Whitney-U and Chi-square test were used to compare patient characteristics between patients listed and patients not listed. Reproducibility of the SALD system was evaluated using intraclass-correlation coefficients (ICC) and Bland-Altman plots. Correlations between CT scores (listed patients only) and PFTs were investigated using Spearman's correlation coefficients (r_s). To estimate survival, it was a priori decided to group LAS and CT scores into tertiles. Using these tertiles, Kaplan Meier curves were constructed and compared using Cox regression. Primary endpoint was death on the waiting list. Each patient was included from listing up to LTX, death, or end of follow up. Patients were enrolled up to December 31 2005, the census date was December 31 2006. Survival differences between centers were evaluated by dividing the centers into 6 groups according to geography, as some centers did not contribute enough patients for separate analysis (patient characteristics: online supplement, Table E2). LAS was calculated only for patients ≥ 12 years ($n = 334$) (29), as pediatric lung allocation is still based on waiting time.(8) Multiple imputations were used to estimate missing clinical data needed in the calculation of the LAS scores (online supplement). Multivariate Cox regression models were used to evaluate the predictive value of the SALD and imputed LAS scores. Goodness of fit was assessed using log-minus-log plots and the Therneau-Grambsch proportional hazards test. Sensitivity analysis was performed to evaluate the influence of missing data on the association of LAS with survival (online supplement). SPSS version 15.0 and STATA version 11.1 were used for statistical analyses. Results are displayed as median (10th – 90th percentile) unless indicated otherwise. $P < 0.05$ was considered significant.

Results

Study population

From the 565 eligible patients, 154 subjects were excluded, resulting in 411 patients (Figure 1). For patients with multiple screenings, we only included the last screening before listing. Subsequently, 366 (89%) patients were listed for LTX after screening, and consisted of 247 (68%) adults, 87 (24%) adolescents aged 12-18 years, and 32 (8%) children younger than 12 years. Patient characteristics are described in Table 1.

Part 3 Further validating CT as surrogate endpoint in CF

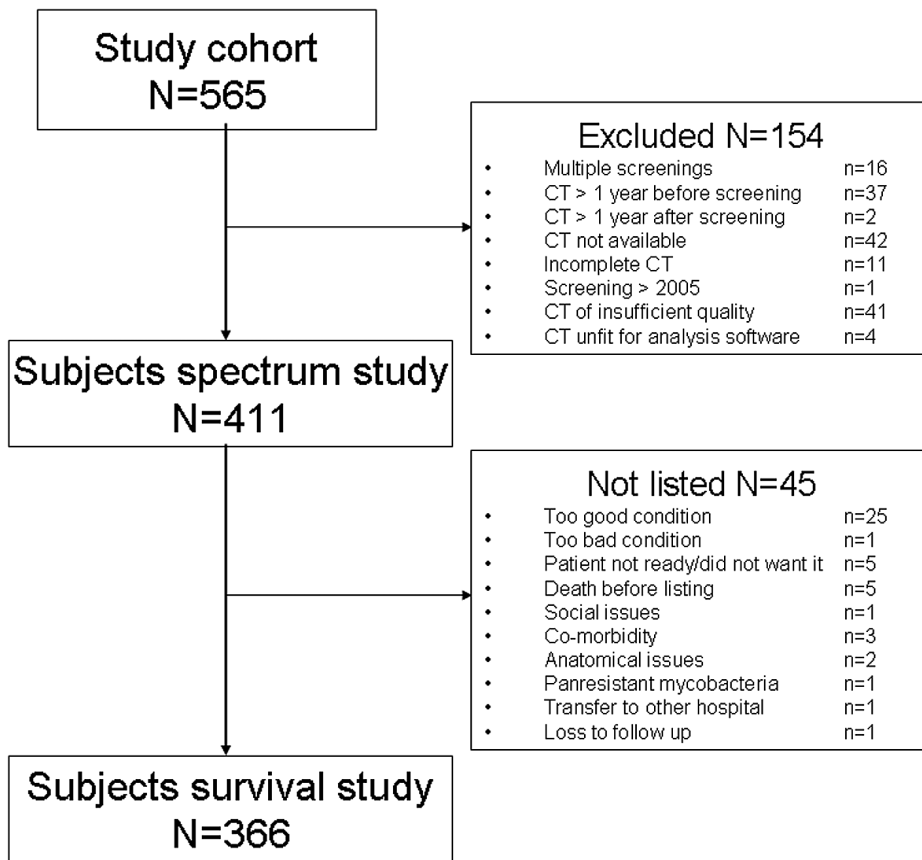


Figure 1. Flow chart of the study cohort.

Within observer agreement for the SALD system was good, ICC for the 4 components ranged from 0.92 to 0.97. Bland-Altman plots showed that differences in scores were independent of the magnitude of the scores (Online supplement, Table E3, Figure E2).

SALD spectrum

We identified a SALD spectrum ranging from predominantly infection/inflammation to predominantly air trapping/hypoperfusion. The distribution of the SALD components for individual patients is visualized in Figure 2.

Correlation SALD CT scores – PFTs

Weak correlations were found between INF and FVC ($r_s = -0.18$ $p=0.001$), AT and FEV₁ ($r_s = -0.23$ $p<0.001$), and NOR and FVC ($r_s = 0.11$ $p=0.04$) as well as FEV₁ ($r_s = 0.28$ $p<0.001$).

Table 1. Patient characteristics and CT scores for the study cohort (n=411), divided in patients who were listed (n=366) and patients who were not listed (n=45) for lung transplantation after screening. The n indicates the number of observations on which the estimates are based per parameter, the p-values indicate the significance of differences between the groups.

Parameter	Patients listed (n=366)	n	Patients not listed (n=45)	n	p-value
Males	186 (51%)	366	29 (64%)	45	0.11
Age (years)	22.8 (13-38)	366	20.8 (11-39)	45	0.32
BMI (kg/m ²) [†]	18 (15-22)	356	18 (15-22)	43	0.62
Pancreatic insufficiency	335 (93%)	359	42 (96%)	44	0.44
Diabetes mellitus	132 (37%)	358	9 (21%)	44	0.04
P. aeruginosa	316 (89%)	357	36 (88%)	41	0.80
B. cepacia complex	24 (7%)	355	3 (7%)	41	0.75
FEV ₁ (% predicted) [‡]	25 (16-37)	356	29 (20-51)	44	0.001
FVC (% predicted) [*]	42 (28-59)	340	48 (30-69)	40	0.004
Corticosteroid use	128 (36%)	353	12 (27%)	44	0.32
Oxygen use	282 (84%)	336	23 (53%)	43	<0.001
6 minute walk distance (meters)	431 (280-588)	288	440 (248-600)	38	0.92
PCO ₂ [§] (kPa)	6.1 (5.1-7.8)	318	5.9 (4.7-7.9)	37	0.11
PO ₂ [#] (kPa)	8.0 (5.7-10.0)	318	8.3 (5.6-11.3)	37	0.54
Waiting time (days)	237 (30-758)	366	-	-	-
LAS [§]	34.9 (32.7-38.6)	334	-	-	-
SALD [¶] scores (%)					
Infection/inflammation	29 (18-42)	366	27 (16-36)	45	0.02
Air trapping/hypoperfusion	50 (35-62)	366	49 (36-62)	45	0.82
Bulla/cysts	0 (0-1)	366	0 (0-2)	45	0.83
Normal/hyperperfusion	19 (12-31)	366	22 (14-36)	45	0.007

Data are given as patient numbers (%) or median (10th – 90th percentile).

[†] Body Mass Index

[‡]Forced expiratory volume in 1 second

^{*}Forced vital capacity

[§] Carbon dioxide partial pressure

[#] Oxygen partial pressure

[§] Lung allocation score (only calculated for children ≥ 12 years of age)

[¶] Severe advanced lung disease

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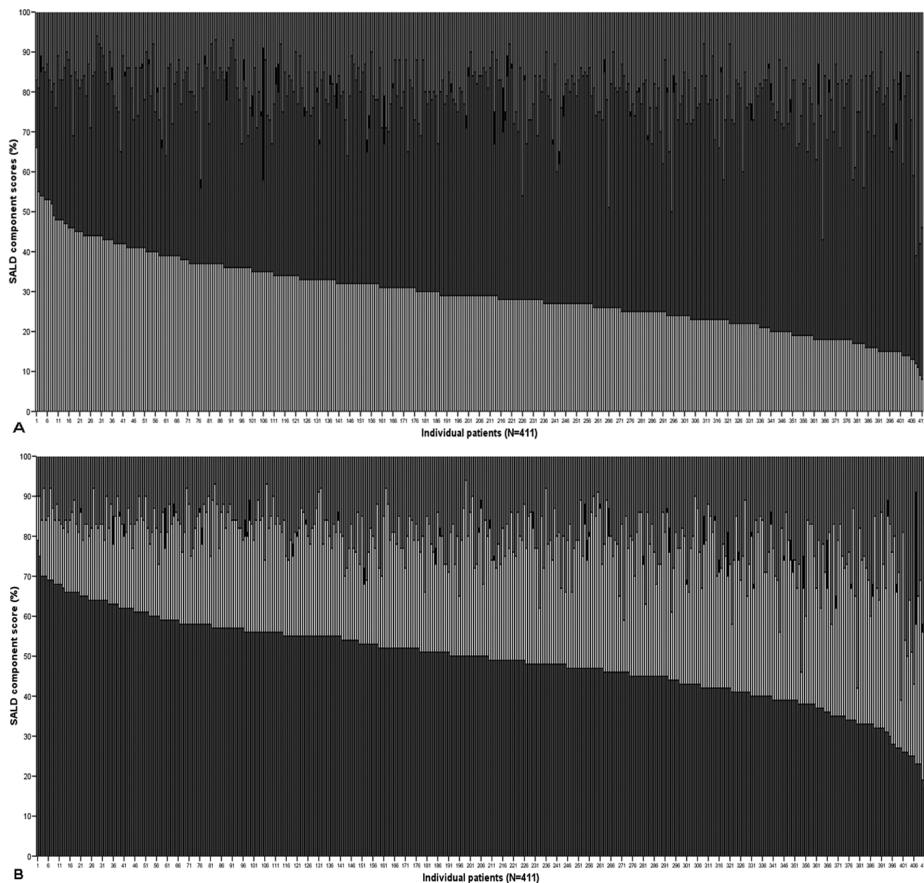


Figure 2. Visual distribution of the 4 categories of the SALD CT scoring system in the 411 patients who were screened for lung transplant: white = infection/inflammation (INF); dark grey = air trapping/hypoperfused (AT); light grey = normal/hyperperfused (NOR); black = bullae or cysts (BUL). Patients are sorted according to their infection/inflammation component (A) or air trapping/hypoperfusion component (B). A full color version of this image can be found on page 218 in the color section

Survival analysis

Of the 366 listed patients, 67 (18%) patients died while waiting, 263 (72%) patients underwent LTX after a mean waiting period of 10 months, and 36 (10%) patients were still awaiting LTX after a mean waiting period of 20 months. No patients were lost to follow up. All deaths were due to cardiorespiratory disease. Waiting list survival did not change with calendar year of listing. Minor differences in survival were found between centers ($p=0.028$), but after adjustment for LAS no significant differences remained ($p=0.84$). No survival differences were found between children younger than 12 years and the adults and adolescents ($p=0.43$). Kaplan-Meier survival curves for INF and LAS, both variables categorized into tertiles, are

shown in Figure 3. These univariate analyses showed significantly worse survival with higher scores, with $p=0.001$ and 0.012 for INF and LAS, respectively. Simultaneous evaluation of both variables using Cox regression showed that INF had predictive value for survival on top of LAS ($p=0.003$, Table 2). Further analysis showed that the effect of INF did not depend on the levels of LAS (interaction p -value= 0.83). Similar results for tertiles of INF were found when the Cox-model additionally allowed for center. LAS and center adjusted hazard ratios of INF tertiles 2 and 3, both in comparison with the lowest tertile, were 1.57 ($p=0.24$) and 2.53 ($p=0.008$), respectively. Additional analysis investigating the use of INF and LAS as continuous variables are provided in the online supplement.

Evaluating NOR, worse survival was found with decreasing scores. Three-years Kaplan-Meier survival for tertile 1 was 70%, and decreased to 50% for tertile 3 ($p_{\text{trend}}=0.003$). However, NOR did not add significantly to the Cox model including INF and LAS ($p=0.18$). Sensitivity analysis showed no influence of missing data on the association of LAS with survival (online supplement).

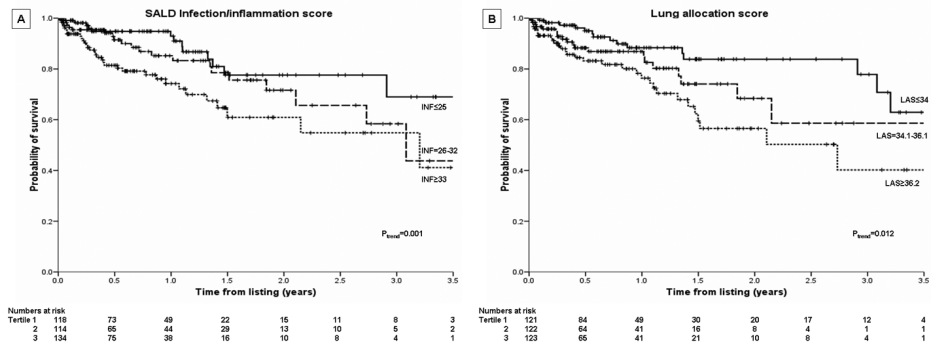


Figure 3. Kaplan-Meier plots of the proportion of cystic fibrosis patients alive while awaiting lung transplantation. Individuals are grouped in tertiles of SALD infection/inflammation (INF) score (plot A) and the mean Lung Allocation Score (LAS) (plot B). The full cohort of 366 patients was used to construct plot A, however, children < 12 years of age ($n=32$) were excluded from plot B, as LAS scores for this cohort cannot be generated. Tertiles for INF (%) are as follows: tertile 1 ≤ 25 (solid line), 2=26-32 (dashed line), 3 ≥ 33 (dotted line), and for LAS: tertile 1 ≤ 34 (solid line), 2=34.1-36.1 (dashed line), 3 ≥ 36.2 (dotted line).

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Table 2. Hazard ratios (HR) derived from the multivariate Cox model with the 95% confidence interval (95% CI) according to tertiles of the Lung Allocation Score (LAS) and SALD infection/inflammation score (INF). The first tertile is used as reference category.

Parameter	HR	95% CI	p-value
LAS			
≤34	1*	-	-
34.1 – 36.1	1.42	0.63 – 3.24	0.40 [#]
≥36.2	2.32	1.17 – 4.60	0.016 [#]
INF (%)			
≤26	1*	-	-
26-32	1.62	0.78 – 3.36	0.19 [§]
≥33	2.65	1.35 – 5.20	0.005 [§]

*Reference category

[#]Lung allocation score p-value for trend=0.013

[§]SALD infection/inflammation score p-value for trend=0.003

DISCUSSION

To our knowledge, this is the first study to investigate the association between CT findings and survival in CF. Several studies have aimed to identify reliable predictors of survival, but CT-related parameters have never been included in survival analyses.(10-12, 17, 30-31) In the current study, we found an association between CT and survival. This is a clinically important finding, as it implies that CT parameters can improve survival prediction models, such as the LAS. The LAS was introduced to reduce wait list mortality by prioritizing candidates based on urgency.(8) LTX candidates are ranked according to their LAS, which is calculated using risk factors associated with mortality before and after transplantation. Early results from the introduction of the LAS are encouraging; decreases in the number of patients awaiting LTX, as well as in waiting time and mortality have been reported.(32) To date, only clinical parameters are incorporated in the LAS. We showed that INF had independent predictive value to LAS, strongly suggesting that CT may improve the predictive value of LAS for CF. As supportive evidence, the prognostic value of CT has previously been shown for idiopathic pulmonary fibrosis (33-34), and CT is currently incorporated in these patient's transplantation guidelines.(18) Whether CT adds to the predictive value of LAS in other lung diseases warrants further investigation. Another consideration is that our multivariate model only included LAS and INF, due to the limited number of events. Analysis including INF and the separate LAS parameters in a larger group is necessary to evaluate which components of LAS are of minor influence when the influence of INF is allowed for.

In this study, we calculated LAS for patients listed between 1990 and 2005. However, the LAS was introduced in May 2005. Thus, most patients were listed in the pre LAS era. Furthermore, patient selection was based on the 1998 ATS guidelines which were updated in 2006.(18) In addition, patient survival has improved over the years.(35-37) Although we found no trend for improved survival with listing year, these factors may affect the generalizability of our results to patients currently awaiting LTX. Future studies are needed to address these concerns.

In this study, the possibility of informative censoring exists, where the reason for removal from the list for LTX is not independent of the outcome. However, sensitivity analysis using the composite endpoint “death or LTX” showed that INF remained significantly correlated with survival ($p=0.039$), suggesting that informative censoring did not greatly affect our results.

The correlation between CT and survival is an important step in the validation of CT as surrogate endpoint in CF. Previous studies suggested that CT may substantially reduce sample size in clinical trials, increasing the feasibility of such trials.(38) CT has already been linked to pulmonary exacerbations (25-26), and quality of life.(27) The association with survival was an important missing link. Often, new therapies aim to increase survival, and surrogate markers of survival, such as FEV_1 , are frequently used as endpoint. Our results support the use of CT as surrogate endpoint, as it shows that CT can predict survival.

The spectrum of structural abnormalities found in this study confirms the results of our pilot study that INF and AT are important components of SALD.(28) This observation is important for prevention and treatment of SALD. Therapeutic strategies for patients with predominantly INF probably need to be different from that of patients with AT. Tailored treatment of the SALD subtypes at an earlier stage may potentially reduce mortality and improve the quality of life. Further investigation is warranted.

We found weak correlations between SALD scores and PFTs. These results are in agreement with findings in mild to moderate CF.(22, 39) INF was not correlated with FEV_1 , an important screening criterion in CF.(18) FEV_1 correlated weakly with AT and NOR, which showed no substantial predictive value. This strengthens the concept that CT can contribute relevant information to survival models independent of PFTs.

This study has some limitations. First, data was collected by a single investigator during site visits. This minimized workload for the centers, reduced variability in data collection, and minimized missing data. However, no re-sampling was possible to check for errors. Nevertheless, we considered errors in follow up to be uncommon, as this was robustly divided in death, LTX, or alive. Second, SALD

scoring was performed by one observer. Thus, interobserver variability could not be assessed. However, importantly, intraobserver agreement was very good. Given the analysis time, our system requires automation to be feasible in clinical practice. To date, we have developed a framework to classify the SALD components and showed that these automated INF scores correlated well with our semi-automated scores.(40) Next step will be to investigate the association between automated scores and survival. Third, CTs were made with different scanners and protocols. This may have introduced some bias related to differences in resolution and density distribution. However, we consider it unlikely that this could have affected the robust division of lung tissue in the 4 SALD components. Image quality was assessed before scoring. In general, manual systems are generally considered less sensitive to differences between CTs and protocols.(38, 41) Fourth, United Network of Organ Sharing default values were used for pulmonary artery systolic pressure and carbon dioxide partial pressure increase. However, we consider it unlikely that this affected the association between LAS and survival, as these defaults were normal or least beneficial values and therefore unlikely to contribute to a higher LAS than expected when using true values.

In summary, in this study we found a significant association between CT parameters and survival, with independent predictive value of CT to LAS. This strongly suggests that in CF, CT can add significant information to survival prediction models. In addition, we reported a spectrum of abnormalities which may warrant more personalized clinical management of CF patients with SALD.

Acknowledgements

We would like to thank Marcel Koek for designing the software tool used to assign the SALD scores in this article. We would like to acknowledge Prof. Dr. M. G. M. Hunink for the useful discussions on the design of the study and the critical reading of the manuscript. In addition, we would like to acknowledge Dr. C. J. Gries for her input on the LAS calculation.

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Screening data and definitions

The following data was collected, using the following definitions:

Age - age at the time of the CT scan

CF diagnosis - clinical features typical for CF, positive sweat test, and/or the presence of two CF mutations

Pancreatic exocrine insufficiency - need for supplemental pancreatic enzymes

CF-related diabetes - need for subcutaneous insulin or oral medication

Corticosteroid use - use of oral corticosteroids at the time of screening

Oxygen - supplemental oxygen at home at the time of screening, expressed in liters per minute and specified for the time of day used.

Assisted ventilation - Need for assisted ventilation at the time of screening (yes/no) If yes, then the type of ventilation (BiPAP, CPAP, mechanical ventilation) and time of use (continuous/intermittent) was recorded.

BMI - weight at the time of screening in kilogram/(length at time of screening in meters)²

Microbiology - Presence of a given micro-organism in sputum culture at the time of screening

Six-minute walk distance - distance walked in 6 minutes in meters, tested at the time of screening

Blood test results - creatinine, blood gas analysis

Spirometry - spirometry values performed or stated at the time of screening expressed in percent predicted. Recorded were forced expiratory volume in one second (FEV₁), and forced vital capacity (FVC).

Standardized CT scoring procedure

All scans were evaluated using a 10x10 mm grid. This grid size was selected by visually evaluating different sizes to see which one would capture the abnormalities best using the slice at carina level in 10 randomly selected scans. Since the original spacing between slices for all scans ranged between 3 mm and 10 mm, we planned to use 10 mm spacing as default for all scans. However, analysis of a scan with 10 mm spacing (approximately 24 slices) took 90 minutes to complete. To reduce analysis time, we investigated the effect of increasing the spacing on the SALD scores. First, we scored 25 randomly selected scans using 10 mm spacing. Second, we increased the spacing by deleting slices to create a spacing of 20 mm, 30 mm and 40 mm. Third, we assessed the agreement between the SALD scores derived from scans with 10 mm spacing and respectively 20, 30, and 40 mm spacing using intraclass correlation coefficients (ICC) and Bland-Altman plots. Excellent agreement for all SALD component scores was shown, with ICC values of 0.99 (10 vs 20 mm), 0.99 to 0.98 (10 vs 30 mm), and 0.98 to 0.96 (10 vs 40 mm). In addition, we assessed individual differences between the 10 mm scores and the scores from 20, 30 and 40 mm spacing. Using a maximum of 5% difference in scores as cut off value, 1 slice every 30 mm spacing was still acceptable. This reduced analysis time to 20-30 minutes per scan. Since this study included pediatric and adult CTs, there was some overlap between the mean number of images per CT for 30 mm (5 to 9 slices) and 40 mm spacing (4 to 6 slices). To ensure enough slices were scored, we set the minimum on 6 slices per scan. If a scan contained less than 6 slices, 20 mm spacing was used.

For film scans, 30 mm spacing was created by manually skipping the required number of slices to reach a spacing closest to 30 mm. For digital scans, the software tool automatically created a subset of the scan with the appropriate spacing between slices.

Before scoring the complete data set, we first established within observer agreement for 30 mm spacing, by re-scoring the 25 randomly selected CTs after one month. Good agreement was shown (ICC range 0.88-0.94), after which analysis of the complete data set was commenced.

For each scan, start and end slice were standardized using the following definitions: the start slice was considered the first slice in which lung tissue was clearly visible for both lungs. The end slice was considered the slice in which both right and left dome of the diaphragm were visible and lung tissue was still visible for scoring. Only lung parenchyma was scored, thus, lung hili, trachea, main right and left bronchi and diaphragm were not annotated. Window level and width were preset at -500/1600. If needed, adjustments were made to increase the visibility of the areas with normal/hyperperfused tissue and air trapping/hypoperfused tissue.

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This was done using slices where the differences between these areas were best visible, which was usually at the basal level.

All scans were scored using a standardized procedure. First, the lungs were semi-automatically segmented using region growing with one manually indicated seed point per lung. This segmentation was used to mark all grid cells belonging to lung tissue, using the label of the SALD component predominantly present in the scan (usually air trapping/hypoperfusion). Second, the observer manually specified per grid cell to which SALD component it belonged by adjusting the label when appropriate. Each SALD component was assigned a different label, shown as a coloured overlay on the image. The SALD components were scored as follows:

Infection/inflammation: all grid cells were scored that contained (parts of) abnormalities belonging to this component (bronchiectasis, bronchial wall thickening, mucus, and/or consolidations). Vessels were considered to belong to normal tissue.

Air trapping/hypoperfusion: grid cells were scored when $\geq 50\%$ filled with tissue with an abnormal hypodense aspect.

For normal/hyperperfusion: grid cells were scored when $\geq 50\%$ filled with normal or hyperlucent lung parenchyma.

Bulla and cysts: all grid cells were scored that contained (parts of) air spaces with no visible connection to the bronchial tree.

Imputed data analysis

Multiple imputations were used to estimate missing values of functional status ($n=175$), 6 minute walk test distance ($n=78$), liters of oxygen at rest ($n=117$), body mass index ($n=10$), forced vital capacity ($n=26$), partial pressure of carbon dioxide ($n=48$), presence of diabetes ($n=8$), and creatinine ($n=19$). Since pulmonary arterial systolic pressure was not measured in most cases, the United Network of Organ Sharing (UNOS) default value of 20 mmHg was used for all patients. Increase in carbon dioxide partial pressure was not part of the LAS when the study started, thus UNOS least beneficial value indicating no change was used. These components needed in the calculation of LAS scores were imputed using Stata software (version 11.1). Twenty data sets with imputed data for the missing components were constructed and LAS scores were subsequently calculated in each resulting dataset. These resulting LAS scores were grouped into tertiles. Cox regression, taking account of these multiple imputed values for LAS, was used for the evaluation of LAS scores regarding their impact on waiting list mortality. For all other

analyses occasional missing values not needed for calculation of the LAS, e.g. for FEV₁, were disregarded and no imputations were done.

Sensitivity analysis

A large group of patients had missing values for functional status, an important factor in the determination of the LAS. Therefore, we performed a sensitivity analysis to evaluate the influence of missing data for functional status on the association of LAS with survival. Kaplan-Meier plots for LAS derived from multiple imputations were compared with plots derived from LAS calculations using the defaults “No assistance with activities of daily living (ADL)” and “Some assistance with ADL”. Using these defaults for patients with missing values showed that correlations between LAS and survival remained significant.

INF and LAS as continuous variables

The use of INF and LAS as continuous variables gave satisfactory fits in the Cox regression, with hazard ratios for INF (per 10 percentage points increase in score) of 1.45 (95%CI 1.12 – 1.85, p=0.003) and LAS (per point increase in score) of 1.1 (95%CI 1.04 – 1.17, p=0.001).

Table E1. Scanning characteristics for all CT scans included in the study (n=411).

Characteristic		n
Number of identified scanners	31	368
Slice spacing (mm)	10 (0.5-20)	411
Slice thickness (mm)	1.5 (0.8-10)	408
Beam potential (kV)	120 (90-290)	404
Beam current (mA)	150 (28-420)	382
Gantry rotation time (s)	1.0 (0.4-6.8)	377

Numbers given are median (range), or total number.

Not all scanner types and scanning parameters could be identified, therefore the number of number of identified scanners and the number of observations on which the estimates are based is displayed (n).

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Table E2. Patient characteristics for the 17 centers divided into 6 groups according to geography, and the overall p-values.

Parameter	A (n=100)	B (n=78)	C (n=71)	D (n=44)	E (n=43)	F (n=30)	p-value
Males	46 (46%)	35 (50%)	37 (52%)	23 (52%)	26 (51%)	19 (63%)	0.71
Age (years)	17.1 (10-37)	25.9 (15-40)	25.0 (12-39)	22.6 (15-40)	25.0 (14-39)	20.1 (13-36)	<0.001
BMI (kg/m ²) [†]	17 (14-22)	19 (16-22)	18 (14-21)	17 (15-20)	19 (15-23)	19 (15-23)	<0.001
Diabetes	28 (29%)	25 (36%)	30 (46%)	13 (30%)	26 (51%)	10 (33%)	0.054
B. cepacia Complex	3 (3%)	5 (7%)	8 (13%)	1 (2%)	7 (14%)	0	0.025
FEV ₁ (% predicted) [‡]	29 (16-44)	23 (15-35)	24 (16-34)	22 (14-33)	23 (17-31)	27 (17-37)	<0.001
FVC (% predicted) [*]	46 (27-65)	37 (22-51)	39 (29-49)	43 (28-59)	44 (30-58)	42 (29-68)	<0.001
Oxygen use	68 (68%)	62 (89%)	60 (85%)	28 (64%)	47 (92%)	17 (57%)	<0.001
6 minute walk distance (meters)	447 (337-596)	400 (280-552)	380 (144-500)	433 (265-600)	485 (302-628)	454 (299-570)	<0.001
PCO ₂ ^{&} (kPa)	5.6 (4.8-7.9)	6.3 (5.3-8.1)	6.1 (5.3-7.6)	5.9 (4.8-9.1)	6.3 (5.0-8.1)	6.7 (4.8-7.5)	0.011
PO ₂ [#] (kPa)	8.5 (5.3-10.9)	7.5 (5.5-9.9)	7.7 (5.7-9.5)	7.8 (5.1-9.9)	8.4 (6.7-9.9)	8.4 (4.9-10.8)	0.009
Waiting time (days)	284 (18-1169)	304 (50-783)	168 (22-533)	185 (32-545)	287 (34-726)	238 (49-607)	0.07
LAS [§]	33.9 (32.3-38.2)	35.6 (33.0-40.8)	35.5 (34.0-37.5)	34.6 (32.7-37.6)	36.0 (33.4-39.1)	33.9 (32.4-37.0)	<0.001

Data are given as patient numbers (%) or median (10th – 90th percentile).

[†]Body Mass Index

[‡]Forced expiratory volume in 1 second

^{*}Forced vital capacity

[&]Carbon dioxide partial pressure

[#]Oxygen partial pressure

[§]Lung allocation score

Table E3. Intraclass correlation coefficients (ICC) and the 95% confidence interval (95% CI) for the within observer agreement of the SALD CT scores for a subset of 25 scans.

SALD Parameter	ICC	95% CI
Infection/inflammation score (%)	0.97	0.94-0.99
Air trapping/hypoperfusion score (%)	0.96	0.92-0.98
Bulla/cysts score (%)	0.92	0.84-0.97
Normal/hyperperfusion score (%)	0.96	0.91-0.98

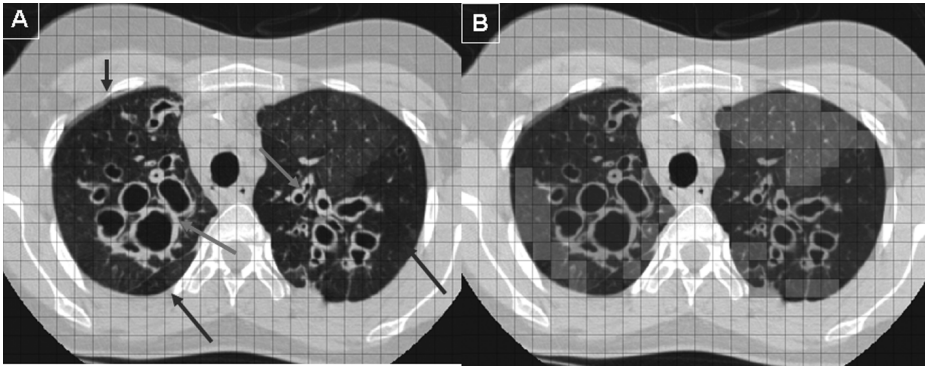


Figure E1. Images show a slice at the level of the mid-trachea of a chest CT scan of a CF patient with SALD. Image A shows the original slice on which the abnormalities are indicated by arrows. Red arrows: bronchiectasis, blue arrows: air trapping/hypoperfusion. Image B shows the same slice after the observer has manually annotated the abnormalities with colours. Red=inflammation, blue=air trapping, green=normal perfused tissue. A full color version of this image can be found on page 217 in the color section

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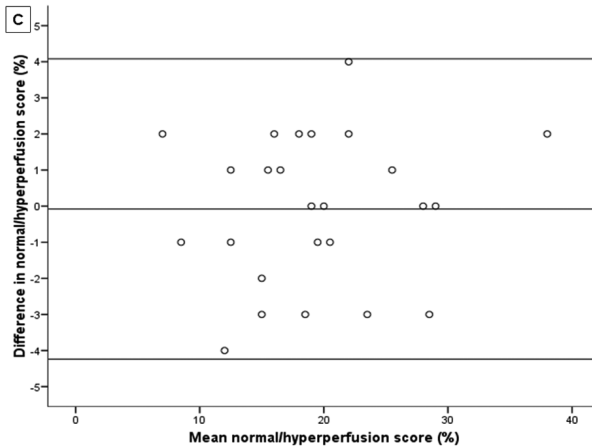
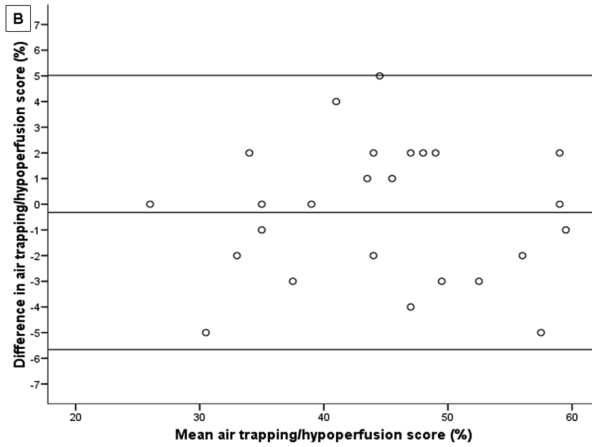
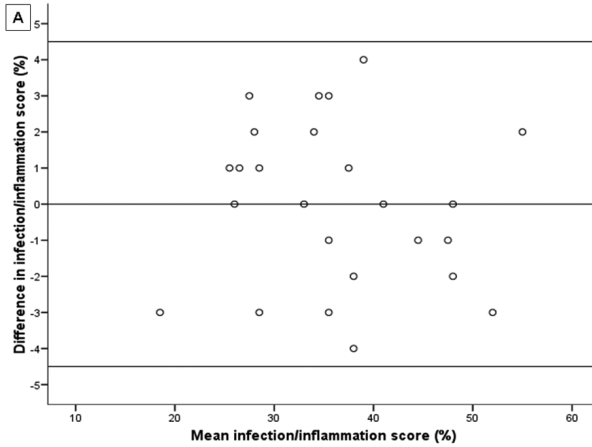


Figure E2. Bland-Altman plots for within observer agreement for the SALD scores infection/inflammation (plot A), air trapping/hyoperfusion (plot B) and normal/hyperperfusion (plot C). All plots show that differences (first minus second measurements) in scores are independent of the level of the scores. The plot for SALD bulla/cyst score is not shown, as a score greater than zero was only present in 1 patient.

Chapter 8

Chest computed tomography;
a validated surrogate endpoint
of cystic fibrosis lung disease?

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HAWM*

Soon to be submitted

Abstract

Clinical trials for the treatment of cystic fibrosis (CF) lung disease are important to test and optimize new therapeutic interventions. To evaluate the effect of these interventions, sensitive and accurate outcome measures are needed. The most commonly used endpoints are spirometric variables such as the forced expiratory volume in one second (FEV_1) and respiratory tract exacerbations (RTE). Unfortunately, these endpoints are relatively insensitive to monitor progression of CF lung disease, and thus require a large number of patients when used in clinical studies. In addition, these endpoints are not suitable to study CF lung disease in young children. Chest computed tomography (CT) holds great promise for use as a sensitive surrogate endpoint in CF. A large body of evidence has been produced to validate the use of chest CT as primary endpoint to study CF lung disease. However, before chest CT can be used in clinical trials, it has to be recognized as a validated surrogate endpoint by regulatory agencies. The aim of this review is to summarize what is currently known about the use of chest CT as surrogate endpoint in clinical trials in CF.

Introduction

Cystic fibrosis (CF) lung disease is the primary cause of death in most CF patients. Thanks to improved treatment, median age currently approaches 38 years.(1) New therapeutic agents such as inhaled antibiotics and disease modifying agents are in development to further improve current treatment.(2-4) To test the effectiveness and safety of these drugs in randomized clinical trials, sensitive and accurate outcome measures are needed. Before an outcome measure can be used as a primary endpoint in a clinical study, it has to meet stringent requirements. First, it must reflect the presence and severity of the disease. Second, it has to reflect clinically meaningful improvement in disease severity when the disease is treated effectively. Third, the measurement must be reproducible. Fourth, changes in the endpoint should closely match changes in the true outcome.(5) Two types of endpoints can be distinguished; clinical or “true” endpoints, and surrogate endpoints. The US Food and Drug Administration (FDA) defines clinical endpoints as direct measures of how a patient feels, functions, or survives, and are thus expected to predict the effect of a therapy. The FDA defines surrogate endpoints as laboratory measurements or physical signs that can be used as a substitute for a clinical endpoint.(6) Surrogate endpoints are generally used when the clinical benefit may not be detectable in trials of reasonable cost, duration, or size.(7) FDA regulations state that a surrogate endpoint is considered to be “reasonably likely to predict clinical benefit and, therefore, useable for drug approval if there is evidence based on epidemiologic, therapeutic, pathophysiologic, or other data supporting the association of the surrogate with the clinical benefit”.(7)

In CF, two endpoints to assess clinical benefit are currently used; respiratory tract exacerbation rate (RTE-R) and quality of life (QoL).(8) RTE-R is an important endpoint that has been shown to increase with age and more severe lung function impairment.(9) In addition, there is a clear association between RTE-R and survival in CF.(10) Unfortunately, the use of RTE-R has disadvantages. It is a relatively insensitive endpoint, especially in (young) children with early lung disease in whom exacerbations due to underlying disease severity are indistinguishable from intercurrent viral lower respiratory tract infections. As a result, RTE-R requires a large sample size when used in clinical studies.(11) Furthermore, there is no universal consensus regarding the definition of an RTE.(12)

QoL is another important clinical endpoint. The FDA accepts patient reported outcome measures as primary or secondary endpoints if they are appropriate for the disease, product, and indication.(13) QoL has the advantage of measuring directly how a patient reports to feel or function. The two most commonly used CF-specific QoL questionnaires (the CF questionnaire-revised (CFQ-R), and the CF Quality of Life Questionnaire) are well validated with demonstrated reliability, validity, and sensitivity.(14-15) The CFQ-R has been used as a primary endpoint

in a study evaluating the effectiveness of an inhaled antibiotic against *Pseudomonas Aeruginosa*.(16) However, further work is needed to standardize the use of QoL instruments in clinical research. Furthermore, the development of new QoL instruments that are sensitive to smaller and earlier changes in symptoms is needed, as improvements in overall health status and lung function are seen in CF birth cohorts.(13)

The most commonly used surrogate endpoint in CF is the forced expiratory volume in one second (FEV₁). The FEV₁ is indirectly related to structural lung damage. When structural damage affects a large volume of the lungs, low values are observed.(17) Thanks to improvements in CF therapy over the last decades, FEV₁ has become relatively insensitive for monitoring progression of CF lung disease. Currently, FEV₁ is in the normal range for most patients until adolescence, and average annual FEV₁ decline is less than 1%.(18) In addition, FEV₁ is insensitive to detect early and localized structural changes (17), is difficult for young children to perform, and is an inappropriate measurement for infants and most pre-school children.(8) Thus, there is a need for new, more sensitive surrogate endpoints in CF that reflect mild lung disease and that can be used to assess lung disease in infants and young children.

A promising endpoint that has been extensively studied since the mid nineties is standardized assessment of images of the lungs obtained by chest computed tomography (CT). Importantly, using chest CT, structural abnormalities can be easily observed. Various methods have been developed to quantify these structural abnormalities. In addition, a large number of studies have been done to validate chest CT as a surrogate endpoint. The aim of this review is to summarize what is currently known about the use of chest CT as a surrogate endpoint in clinical trials in CF. In addition, we will discuss the advantages and disadvantages associated with its use, and the future work needed to further improve sensitivity and accuracy of chest CT for use in clinical trials.

CF lung disease

At birth, CF patients have macroscopically normal lungs.(19) The structural changes related to CF however, occur early. Pathology studies have shown the presence of structural abnormalities such as bronchiectasis (BE), and mucus impaction even in infants with CF aged 0 to 4 months.(20) In addition, all children in this age group were found to have evidence of bronchial wall inflammation. Airway wall thickening, a frequently observed abnormality in CF, has been correlated with airway inflammation.(21-22) In other lung diseases, such as chronic obstructive pulmonary disease (COPD) and asthma, the severity of airflow obstruction is proportional to the severity of airway wall thickening.(21, 23) Similar correlations have been reported in CF. Furthermore, a 3-fold increase in airway wall thickening was found in specimens from CF patients compared with specimens from COPD

patients.(22) In addition, destruction of the epithelial layer and a substantial loss of cartilage have been described in CF.(24-25) All these factors are likely to contribute to the airflow obstruction that is present in CF.(22) To monitor CF-related lung changes in vivo, CT scanning can be used, as it was shown to correlate well to pathologic findings such as BE.(26)

Image analysis

An important condition for the use and validation of chest CT as a surrogate endpoint is that the structural abnormalities can be quantified in a reproducible manner. This can be done using manual (semi-)quantitative scoring systems and/or automated systems.

Scoring systems

For CT scoring, a range of systems is available with proven reproducibility. In a comparative study, within and between reader reproducibility for 5 scoring systems was found to be good with intraclass correlation coefficients of 0.74 and higher. (27) These systems were mainly developed to quantify the abnormalities in mild to moderate CF lung disease. For CF patients with severe advanced lung disease (SALD), a dedicated SALD scoring system was developed.(28) This system uses a quantitative approach to quantify the most important structural abnormalities of CF lung disease. Using a digital grid projected over the CT image, all lung tissue within one grid cell is annotated into one of 4 components. Three components indicate abnormalities: (1) infection/inflammation (including several abnormalities, from which BE is the most important one), (2) air trapping/hypoperfusion; and (3) bulla/cysts. The fourth component consists of normal/hyperperfused tissue. The SALD system has been shown to correlate with the Brody-II system, with acceptable reproducibility between and within observers.

Automated systems

Ideally, automated systems should be used for CT quantification, as they allow more rapid analyses with near perfect intratest reproducibility. Various semi-automated systems have recently been developed. These systems have been shown capable of measuring airway wall thickness and bronchial internal diameter (29-36), and trapped air (TA).(33, 37-38) These automated systems were able to differentiate CF patients from controls (32-33, 35, 37-38) and parameters correlated well with pulmonary function tests (PFTs). (33, 35, 37-38) In addition, automated systems to quantify BE, airway wall thickening and mucus scores have been published, which correlated strongly with visual scores from radiologists.(29-30, 35) For longitudinal follow up, automated systems have been described that allow serial comparisons (31), and matching of airways.(36) Matching was also used to track progression of TA. In this study, two routine expiratory CTs over 2 years were matched, and the proportion of stable, disappeared and new TA was measured. With this novel approach, TA was found to have a stable component.(39)

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Quantitative TA measurements have been used in intervention trials, such as described by Robinson et al.(40) In this 1-year randomized placebo-controlled trial with dornase alpha, 25 children with mild CF were enrolled. They found that the quantitative TA measures could discriminate differences in treatment effects in children with mild CF. Furthermore, these measures were reported to be more sensitive outcome measures than either spirometry or total CT scores. Thus, automated systems are promising, but there are still substantial technical issues to be resolved. For example, the influence on the measurements by factors such as scan parameters, inflation level, effect of heuristic element in some systems on reproducibility and clinical validity of the measurements.(41) Further development is needed before these systems can be used in multi-center clinical trials.

Presence and severity of disease

The first validation requirement for endpoints is that it must reflect the presence and severity of the disease. Currently, chest CT is considered the gold standard for the detection of BE.(42) An important advantage of CT is that it easily allows recognition of the structural lung abnormalities characteristic for CF, such as BE, which are not present in normal subjects. Furthermore, CT can detect abnormalities early in the course of the disease. In recent cohort studies in children with CF diagnosed through newborn screening structural abnormalities such as BE, TA, and mucus impaction could be observed even in asymptomatic infants.(33, 43-45) In addition, in infants and in older children BE was shown to be progressive.(43, 46-47) Furthermore, CT proved to be more sensitive in detecting and monitoring BE and other structural abnormalities than PFTs related parameters (Figure 1). (46-47) Finally, BE and TA are also the most important components of end stage CF lung disease (Figure 2).(28) Other structural abnormalities that can be



Figure 1. Image showing the dissociation between lung function and lung structure assessed by computed tomography (CT). Shown is a slice of a routine CT scan of a cystic fibrosis patient, performed during annual check up when clinically stable. This slice clearly shows bronchiectasis and bronchial wall thickening (white arrows), and mucus plugging (black arrow).

observed on chest CT in CF are airway wall thickening, consolidations and bulla/cysts. An important advantage of CT over PFTs is that structural lung abnormalities can be differentiated in the above described components.

To summarize, there is a large body of data supporting the concept that CT is sensitive to detect the presence and monitor the severity of structural changes relevant for patients with CF starting in infancy into adulthood.

Lung function at the time of the CT however, was normal, with a forced expiratory volume in 1 second of 98%-predicted.



Figure 2. CT slice of a patient with end stage cystic fibrosis lung disease. This CT scan was made during screening for lung transplantation. Lung function estimated by forced expiratory volume at 1 second at the time of the CT was 21%-predicted. Clearly visible are the bronchiectatic airways (white arrows).

Response to treatment

The second validation requirement for endpoints is that it has to show improvement upon successful treatment of the disease. CT scores have been shown to improve with antibiotic treatment for an RTE. Shah et al. prospectively studied 27 adult CF patients, and showed that CT scores for airway wall thickening, mucus, and air fluid levels in BE and centrilobular nodules improved in respectively 2/19 (11%), 6/18 (33%), 2/2 (100%) and 4/11 (36%) of patients in response to treatment.(48) Similar findings were reported for pediatric patients. Robinson et al. prospective studied 17 pediatric and adult CF patients (mean age 17.2 years), and showed that CT total scores and mucus scores were significantly reduced after treatment for an RTE.(49) Brody et al. studied 8 pediatric CF patients (mean age 12.7 years) experiencing a total of 15 RTEs, and showed that CT scores for airway wall thickening and mucus significantly improved after treatment.(50) Even in very young children, these improvements were observed. Davis et al. studied 13 young children with CF (mean age 17 months), and showed that CT total score, combined bronchiectasis-bronchial dilatation score and hyperinflation score significantly reduced after treatment.(51) CT parameters have also been shown to

improve after treatment with rhDNase (40, 52-53) and trends toward improvements were observed when evaluating the effect of tobramycin solution for inhalation.(54) To date, only one long term study used CT diagnosed BE as outcome parameter.(55) Since BE is an irreversible change, such a study should look at the change in BE development. Clearly, when an intervention is able to prevent BE or stop the progression, this would be considered clinical meaningful. In this study, 32 CF patients aged ≥ 6 years with mostly mild lung disease were randomly assigned to receive 3 cycles of 28 days on and 28 days off treatment with nebulized tobramycin solution for inhalation or placebo. They found that the total CT score significantly improved after 28 days. However, no difference in BE scores between the study arms was observed. The authors concluded that the BE score was only marginally useful in assessing the response to treatment. A limitation of this study was that only 5 inspiratory and 5 expiratory CT slices of each subject were analyzed, which has been shown to reduce the sensitivity to detect changes. (56) To date, no other study has been performed to investigate the long term efficacy of antibiotics, mucolytics or disease modifiers on the development of BE or TA. Thus, while the ability of other CT scores to improve with treatment has been clearly demonstrated, long term effect of treatment on the development of BE needs to be further studied.

Standardization

The third validation requirement for endpoints is reproducibility of the measurement. For CT, it is important to standardize the scanning protocol to optimize reproducibility. The most important variable that requires standardization is the lung volume during scanning. Traditionally, in patients of 5 years and above voluntary breath holding is used during scanning. Patients are instructed by the CT technician or by a recorded voice to perform a maximal inhalation manoeuvre, and then hold their breath during scanning to obtain end-inspiratory images. Next, patients are asked to exhale and hold their breath at the end of the exhalation to obtain end-expiratory images. With this technique however, one does not assess whether a patient was able to follow the instruction correctly and hold his breath at the maximum in- and exhalation level. Unfortunately, for many patients and specifically for children, these manoeuvres are difficult to perform. In a pediatric study (mean age 12 years), scan volumes were compared with plethysmographic lung volume measurements prior to scanning. Average inspiratory volume was at an acceptable level of 77% of total lung capacity (TLC). However, this volume ranged from 55% to 106%. Average end-expiratory volume was 86% of functional residual capacity (FRC) and 140% of residual volume (RV), and thus closer to FRC than to RV. This suggests that expiratory manoeuvres are even more difficult to execute for children.(57) Thus, there is a need for better standardization of the breath hold manoeuvres during CT scanning. This volume control can be obtained by using a spirometer combined with instructions by a qualified lung function technician. Prior to scanning, the patient practices the required breathing

manoeuvres coached by the technician and using a spirometer. Next, the patient performs these manoeuvres with the spirometer during scanning, again coached by the lung function technician. (58) The scanning will commence when the required volumes are reached by the patient. The aim of this procedure is to obtain an inflation level near 95% of slow vital capacity (SVT), and an expiration level near 5-12% of SVT(44, 59) Recently, a new spirometer technique was described; the volume-monitored technique. This method combines coaching with a portable spirometer that generates respiratory tracings to aid the patient in achieving targeted lung volumes.(60) With this method, patients as young as 4 years are able to achieve reproducible images at 95% of full inflation and at 77% of vital capacity for the expiratory images.

In young children, standardization of lung volume during scanning requires a different approach, as most children aged 0-5 years are not able to do a voluntary breath hold at the correct volume level and at the correct moment. Therefore, a non-invasive pressure controlled ventilation (PCV) technique under general anaesthesia or sedation has been introduced.(44) This technique starts off by hyperventilating the child by giving a short series of augmented breaths using positive pressure applied via a facemask to induce a physiologic respiratory pause in the sedated child. During this pause, the lungs are imaged at full inflation by maintaining a positive facemask pressure of 25–30 cm water pressure for inspiratory images, and resting end exhalation by applying no mask pressure for expiratory images.(44, 59) Both spirometer and PCV techniques have been shown to be highly reproducible.(59, 61)

Volume control is particularly important for assessment of BE en TA. In a pediatric study, BE was identified on 30% of images obtained at end-inspiration using PCV techniques compared with 6% of images obtained during quiet breathing. TA was seen in 45% of images obtained at end-expiration compared with 19% of images obtained during quiet breathing.(62) Thus, for standardization of chest CT, volume control is an important condition for both inspiratory and expiratory scans.

Correlation to true endpoints

The fourth validation requirement for endpoints is that changes in the endpoint should closely match changes in the true outcome. The first argument providing that CT meets this criterion is that higher CT scores have been correlated with conditions associated with a more severe course of the disease. For example, higher CT scores were reported for patients with pancreatic insufficiency compared with patients with sufficient pancreatic function.(63) In addition, CT scores strongly correlated with *Pseudomonas Aeruginosa* acquisition, which is a well established risk factor for progressive CF lung disease.(64-66) The second argument that CT meets this criterion, is that CT scores have been linked to the true endpoints RTE-R, quality of life, and survival.

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RTE-R: Two studies have shown that the severity of structural changes on CT correlated significantly with RTE-R.(67-68) The first study described 61 subjects from the Pulmozyme Early Intervention Trial.(67) In this trial, 6- to 10-year-old children with well preserved lung function (forced vital capacity \geq 85%) were included. CTs and PFTs were performed at the beginning and end of the 2-yr trial during which RTE-R was recorded. Nine out of 61 subjects experienced a total of 22 RTEs. PFTs and CT scores at baseline showed significant correlations with RTE-R, although none of these variables by itself predicted RTEs with high accuracy.(67)

The second study was performed in an unselected cohort of 115 children and young adults with CF.(68) In this retrospective study, routine chest CTs and PFTs performed during annual checkup were collected with two years' follow up in which RTE-R was recorded. Fifty-one of the 115 subjects experienced a total of 148 RTEs. BE was found to be the strongest predictor of RTEs of all CT parameters, and added significantly to the predictive value of FEV₁.

QoL: In patients with non-CF BE, studies have shown an association between BE and impaired QoL.(69) In a cross-sectional study including 46 adults with non CF bronchiectasis, the relationship between the BE severity and QoL was investigated. CTs were scored with a modified Bhalla scoring system and QoL was measured using the St George's Respiratory Questionnaire. A significant correlation between BE severity and QoL in patients with a more severe bronchiectatic disease was observed.(70) In a recent study, similar results were observed in CF. In this cross-sectional study, the effect of CT scores on QoL was assessed using routine CTs and CFQ-Rs from 72 children and adolescence with CF. CTs were scored using a modified Brody-II scoring system. Significant correlations between the respiratory domain of the CFQ-R and BE and TA scores were found.(71)

Survival: Recently, a correlation between CT and survival has been established. In a multi-center study including 366 CF patients awaiting lung transplantation, CT scans acquired at the time of screening were scored using a semi-automated version of the manual SALD scoring system.(28) SALD infection/inflammation score (including BE) was shown to be significantly correlated with waiting list survival. In addition, BE score added clinically useful, and practical information to the predictive value of the lung allocation score.(72) The correlation with survival is an important addition to the portfolio of CT as an outcome measure.

Correlation to other surrogate endpoints

In addition to correlations with true endpoints, CT parameters have also been linked to the following frequently used surrogate endpoints.

Spirometry parameters

Several studies have shown a correlation between CT scores and spirometry parameters such as FEV₁, which is currently the most extensively validated surrogate endpoint in CF. From these studies we learned that CT scores are more sensitive than PFT parameters in detecting and monitoring onset and progression of CF lung disease.(46-47) Comparing the validation status of FEV₁ to CT-related parameters, it can be concluded that most of the validation steps for CT have been addressed (Table 1).

Table 1. Overview of the literature published on the validation of the surrogate endpoints FEV₁ and CT.

Parameter	FEV ₁	CT
	References	References
Presence and severity of CF lung disease	(17), (93), (94), (95), (96), (97), (18), (98), (99), (100), (101), (102)	(42), (28), (33), (43), (45), (103), (47), (95), (104), (103)
Response to treatment in CF	(105), (106), (107), (49), (40), (108), (109), (110), (111), (112), (113), (114), (115), (116), (117), (118), (119), (120), (121), (122), (123), (124), (125), (126)	(48), (49), (50), (51) (52), (53), (40), (55)
Reproducibility of the measurement	(127), (128), (129), (130), (131), (132), (133), (134), (135)	(27), (28)
Link to true outcomes in CF		
Respiratory tract exacerbations	(68), (67), (9), (136), (105), (137), (96)	(68), (67)
Survival	(10), (138), (139), (140), (141), (142), (101), (136), (143), (98), (144), (145)	(146)
Quality of life	(147), (148), (149), (150), (151), (152), (153), (154), (155), (156), (157), (158), (159)	(69), (71)

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Lung clearance index (LCI)

CT scores have also been shown to correlate with LCI, a promising marker of early disease derived from multiple breath washout.(73-75) The LCI has been shown to be more sensitive to detect lung disease than spirometry, (73, 76-79). In addition, LCI is reproducible (76, 79), and can be performed in infants.(80) Its narrow range in normal subjects makes it a suitable measure for long term follow up.(73, 76-79) A normal LCI has even been suggested to exclude structural changes on CT (73-74), a finding that was not supported by another study.(75) Its sensitivity to monitor progression of advanced lung disease however, has not been investigated to date. In addition, LCI has not been well validated against other true and surrogate endpoints.

Inflammatory parameters

The severity of structural changes on CT has been shown to correlate with inflammatory parameters in bronchoalveolar lavage (BAL). In a study in 17 infants and young children under the age of 4 years, regional distribution of airway disease was assessed using CT scans, and abnormalities were correlated to markers of lower airway inflammation provided by BAL. They found that in the lobe with greatest disease as indicated by CT, inflammatory markers were higher than in lobes with least disease.(51) These findings have been confirmed in two other studies in infants with CF identified by newborn screening who underwent BAL and CT scanning as part of an early surveillance program.(43, 45)

Thus, CT has been shown to be clearly linked to a number of clinically meaningful outcome measures. These correlations are essential in the validation of CT as surrogate endpoint in CF.

Advantages of using CT in clinical trials

In the previous paragraphs, we have shown that CT meets many of the requirements for surrogate endpoints. CT is able to detect structural changes highly relevant for CF patients, and the abnormalities on CT can be quantified in a reproducible fashion. CT scores can improve with treatment, and standard chest CT scanning procedures have been defined. In addition, CT parameters have been linked to other clinically meaningful outcome measures. Thus, based on these arguments, CT can be considered well validated as a surrogate endpoint for CF-related lung disease. Using CT as surrogate endpoint in clinical studies has the following advantages. First, CT can easily be performed in most CF centers, as virtually all centers are equipped with a CT scanner. Second, CT can be performed across all age ranges, including infants and young children. Third, CT is the most sensitive tool to detect early and regional disease. Compared with spirometry parameters, CT is more sensitive to detect and monitor disease progression.(46-47) It has been estimated that the better sensitivity of CT relative to PFTs can reduce sample size in clinical studies substantially.(63, 81) This would increase the feasibility to run clinical tri-

als in CF. A three-year randomized controlled multi-center intervention study in infants diagnosed through newborn screening using BE diagnosed by chest CT as the primary endpoint is about to start.(82)

Disadvantages of using CT in clinical trials

Clearly, the use of chest CT in clinical trials also has a number of disadvantages such as: ionizing radiation; the need for general anesthesia in young children; costs; and the relative complicated procedures needed for the pressure controlled volume scanning.

Radiation

CT exposes patients to ionizing radiation, which increases one's natural life-long risk of cancer. Children are particularly at risk, as tissue and organs are still growing and developing and therefore more sensitive to radiation effects. In addition, children have a longer life expectancy and therefore more time to manifest the oncogenic effects of radiation. Furthermore, children have a smaller cross-sectional area compared with adults, resulting in a higher radiation dose when scanned with the same protocol.(83) At high exposures, the risk of cancer increases linearly with increasing dose. The relationship between radiation exposure and cancer risk from low-dose radiation (such as CT scanning) is less clear. In a study using a computational model, bi-annual chest CTs (mean dose 1 milli Sievert (mSv)) were shown to carry a low risk of radiation-induced mortality, with reported cumulative cancer mortalities of 1% at age 40 and 6% at age 65.(84) However, with overall survival increasing for CF patients, life-long radiation exposure also increases, and thus the risk of radiation-induced mortality can become more meaningful. Therefore, protocols should aim to limit radiation to the absolute minimum needed to acquire images of sufficient quality. Six slice protocols with a mean dose of 0.19 mSv have been described, and were found to be appropriate for evaluating BE in pediatric patients.(85) However, limited slice CT reduces the ability to identify specific areas and their interval change, thus decreasing its ability to detect a therapeutic effect.

Volumetric CT scanning will improve the ability to identify and compare specific structures on serial CTs. Currently, we can acquire volumetric CTs (inspiratory plus expiratory images) with a mean total effective dose near 1 mSv.(57) This is comparable to 1/3 of the annual US background radiation.(83) These doses can likely be further reduced in the near future.(57) Keeping the risk-benefit ratio of clinical trials in mind, CT should be considered as surrogate endpoint in studies aiming to slow the progression of CF lung disease.

Sedation/anesthesia

Second disadvantage is that CT scanning in infants and young children often requires sedation (i.e., with chloral hydrate) or general anesthesia. These agents are

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very effective, with reported sedation failure rates of less than 1% using chloral hydrate.(86) In addition, they have a good safety profile. In two large retrospective studies reviewing records of children who underwent sedation for imaging studies, adverse events were reported in 0.85% and 0.42% of patients respectively. (86-87) Thus, the risks of sedation are low, but not zero. To minimize these risks, patients should be carefully selected and monitored (88), and sedated using the most suitable technique.(89) The advantage of sedation or anesthesia is that inspiratory lung volume can be standardized and the volume of gas trapping can be determined at end expiration. Sedation or general anesthesia can be avoided using recently developed ultra fast CT scanners. A comparative study showed that excellent image quality could be achieved in infants and small children using a second generation (2 x 128 slices) dual source CT without sedation with a radiation dose similar to that of conventional CT scanners.(90). However, CTs acquired during free breathing are usually taken at a volume level near FRC. Whether the sensitivity and accuracy of these scans to detect structural abnormalities is comparable to that of volume controlled inspiratory and expiratory scans has not yet been investigated.

Costs

Third disadvantage of CT is the high costs of the procedure. Including CT as surrogate endpoint will increase the costs of an intervention study. However, CT likely reduces the required sample size of a clinical trial, which ultimately reduces the total costs of a trial. To the best of our knowledge, the costs and cost-effectiveness of chest CT in CF clinical trials has never been investigated. More research is needed to investigate this further.

Volume control

A fourth disadvantage is that CT protocols including PCV techniques or a spirometer are relatively complicated and require a well trained team. Children of 5 years and older need to be trained and coached by a lung function technician before and during scanning. In addition, a spirometer is required for use in the CT room. Procedures involving children below the age of 5 require personnel experienced in both pediatric sedation/anesthesia and the CV procedure. Furthermore, adequate time slots must be available to execute the protocol. This may limit its feasibility in multicenter trials.

Future research

In this review, we have presented a large body of evidence supporting the use of chest CT as surrogate endpoint in clinical studies. However, even though there is sufficient evidence to support its use in clinical trials in the near future, further improvements can be made that will increase the accuracy and sensitivity of the technique.

Calibration, standardization

CT already has shown its feasibility in multi-center trials.(64, 67, 91) These trials demonstrated the importance of defining a detailed protocol at the start of the study to improve compliance, and to document the ability of the centers to apply this protocol.(92) CT studies require well defined standard operating procedures to guarantee optimal image resolution and the use of the correct reconstruction algorithms. Since CT technology is moving fast, the procedures will require frequent updating.

Image analysis

Standardization is also important for CT quantification. Agreement on the most appropriate scoring systems for the different CF cohorts (infants, children and adults) is necessary. To date, no validated automated image analysis systems are available to quantify for example BE and TA. Such systems can further improve the sensitivity and accuracy of chest CT as a surrogate end point.

Further validation steps

An important next step will be to demonstrate that the effect of an intervention on the CT score predicts the effect on true clinical endpoints such as RTE-R and QoL. This validation is specific to the class of intervention, and the CT score studied. Different CT scores may capture different types of structural changes, and therefore may affect different primary endpoints.(81) Furthermore, it is important to establish the minimal clinically relevant changes for each of the various CT scores. Thus, more comparative studies are needed. In addition, more longitudinal studies will help to further establish the trends of the different CT features over various time periods. This will help defining the required time span for intervention studies. Ideally, a profile should emerge defining the therapeutic indications and target populations where CT will be most useful and likely to show change.

Summary

In this review, we showed that CT scores meet all critical requirements for surrogate endpoints. CT is able to detect structural changes highly relevant for CF patients using standardized image acquisition protocols, and the abnormalities detected using CT can be quantified in a reproducible fashion and can improve with treatment. In addition, CT variables have been linked to a number of clinically meaningful outcome measures. CT can be performed across all age ranges, is the most sensitive tool to assess mild and regional disease, and may reduce sample size requirements for clinical studies compared to other recognized endpoints. The disadvantage of chest CT in clinical studies however, include the radiation exposure to the patient, the need for sedation in small children, high costs, and the more complicated procedures when volume control is needed. Further inno-

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vations in image analysis techniques will help to further improve sensitivity and accuracy of chest CT to monitor CF lung disease. Remaining challenges in the use of CT as surrogate endpoint in clinical trials comprise standardization of CT protocols, procedures and quantification, better assessment of accuracy, and reliability of CT scores, better understanding of the association of CT scores with clinical outcomes, together with assessment of its feasibility in multi-center settings.

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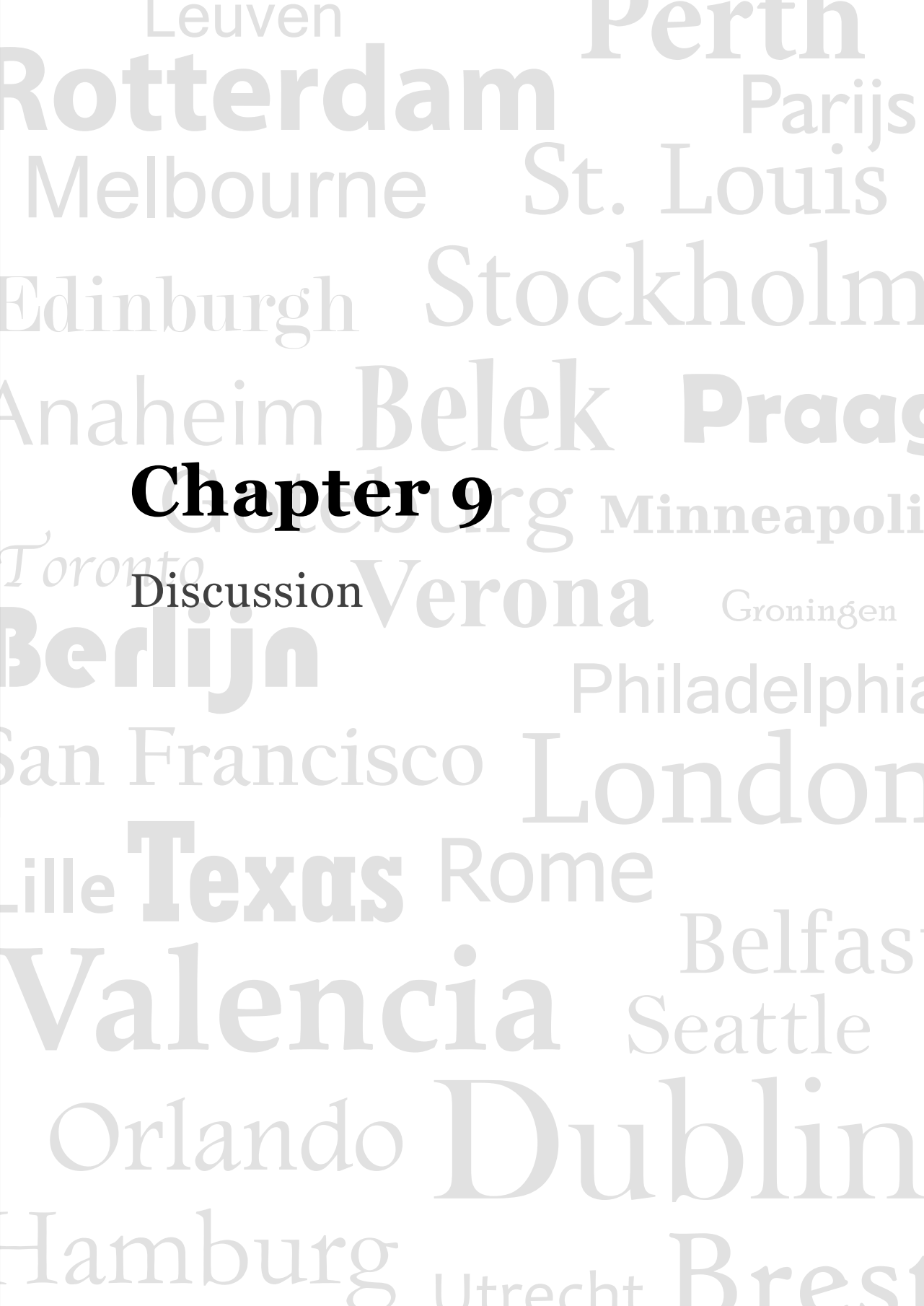
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Chapter 9

Discussion

In this thesis, we aimed to optimize CT protocols and image analysis techniques, and further validate chest CT parameters as surrogate endpoints for CF lung disease in clinical management and clinical trials. In this chapter, we discuss the main findings of our studies in the context of current literature, their implications, and directions for future research.

The first important finding of our studies is that expiratory scans may suffice for assessment of structural changes in CF lung disease. In the study described in chapter 2, we compared CT scores of low dose inspiratory and ultra low dose expiratory scans after a voluntary breath hold, and found that these matched closely. This is important, as exclusion of the inspiratory scan from the routine CT scanning protocol in CF can reduce radiation dose by up to 75%. The CT protocol for the inspiratory scan used in this study exposed patients to a mean effective dose of 0.69 mSv while the exposure for the expiratory scan was only 0.35 mSv. Limiting chest CT scanning to only expiratory images would expose patients to a radiation dose comparable to only 1/8th of the annual background radiation dose (3 mSv) in the United States of America.(1) Reducing radiation dose in CT scanning is important, as CT has been shown to be an increasing source of radiation exposure. (2) However, before we can recommend the use of only expiratory images to assess the structural changes in CF, the following issues need to be further investigated. First, we need to ascertain that the sensitivity of this restricted expiratory protocol to track disease progression is similar to that of the protocol including both the inspiratory and expiratory CT. Currently, disease progression has only been reported for inspiratory images.(3-4) Second, we only included children aged 6-20 years with mild to moderately severe lung disease in this study. Thus, whether expiratory images alone may be sufficient to assess disease onset and progression in infants with early CF lung disease or in adults with more advanced lung disease needs to be further investigated. Third, the effect of inflation level on the assessment of structural CF-related changes should be assessed. In our study, we used voluntary breath holds during scanning. However, current practice in our clinic is to use a spirometer-controlled protocol to standardize inflation levels.(3) Inflation levels influence the detection of airway dimensions. Thus, future studies preferably using spirometer-controlled CTs are required.

The second important finding of our studies is that the number of CT slices can affect the sensitivity of the assessment of trapped air (TA) in CF, and depends on the type of scoring system used. Our study showed that mean TA Brody-II scores from 3-slice protocols were significantly lower compared with mean scores from volumetric protocols. This difference was in the order of 10%. To date, the minimal number of slices required for adequate TA assessment on CT is unknown, and a 3-slice protocol is often used to reduce radiation exposure.(6-14) Our data suggests that these 3-slice protocols tend to underestimate the severity of TA in CF when measured with the Brody-II scoring system. Other studies in CF (4) and

lung transplant recipients (5) also show that limited slice protocols decrease the precision of TA assessment. However, mean TA scores from 3-slice protocols derived from another quantitative scoring system were not significantly different from mean scores from volumetric protocols. Thus, the quantitative scoring system appeared to be less sensitive to the effect of limited slice protocols. This suggests that the effect of the number of slices depends on the type of scoring system used. However, sample size in this pilot study was small. Larger studies preferably using a range of different scoring systems are needed to investigate this further.

The third important finding of our studies is that there is a wide spectrum of structural abnormalities in CF patients with severe advanced lung disease (SALD).

This spectrum ranges from predominantly infection/inflammation-like changes to predominantly air trapping/hypoperfusion-like changes. This has the following clinical implications. First, it shows which abnormalities lead to SALD and thus, should be prevented. Infection/inflammation, which includes bronchiectasis, is an important disease component in SALD. The importance of bronchiectasis in CF has been well recognized.(26-28) Hence, prevention of bronchiectasis is an important treatment target in patients with SALD. The observation that air trapping/hypoperfusion makes up an important volume proportion in SALD is new and offers new opportunities for intervention and prevention. To date, the clinical significance of TA has never been systematically studied. To some extent we can conclude that small airways disease has been neglected as a potential target for treatment. In mild to moderate CF lung disease, there is substantial evidence that TA can be treated to some extent.(6),(7) Thus, TA may be reversible when treated early. Our observation warrants future intervention studies with the aim to target small airways disease more aggressively at an early stage of disease. The other important implication of our findings is that the CT spectrum in SALD offers the opportunity for a more personalized approach for these patients with SALD. Therapeutic strategies for patients with predominantly infection/inflammation are likely different from strategies needed for patients with predominantly air trapping/hypoperfusion. More personalized treatment of the SALD subtypes at an earlier stage may potentially reduce mortality and improve the quality of life.

The fourth important finding of our studies is that TA on CT has a stable component over time, reflecting irreversible small airways damage. This was found in a study in which we investigated the volume and the distribution of TA over time using newly developed image analysis software. By overlaying a baseline and follow up CT of the same subject, we were able to assess whether TA on the follow up CT was stable, new or reduced compared to the baseline CT. Matching has previously been used to track changes in regional bronchial morphology (8), but never to estimate the reversibility of TA. Our observation that at around one third of the small airways disease that causes TA is irreversible has important implications

for the treatment of TA. Dornase alpha has been shown to reduce TA in mild to moderate CF lung disease.(6) Our technique enables visualization of the distribution TA. This information can be used to determine the optimal settings for modern smart nebulizers.(7) Next, the therapeutic effect of the inhaled medication can be visualized. This can be of great value for both clinical practice and clinical trials aiming to reduce TA. TA is present early in the course of CF lung disease (9-10), and forms a substantial component of the abnormalities seen in SALD.(11) Thus, early treatment and monitoring of TA may prevent progression to end stage lung disease. However, our proposed technique requires further improvement, and validation studies using larger number of patients to establish its importance for clinical practice.

The fifth important finding of our studies is that CT bronchiectasis score is a strong independent predictor of respiratory tract exacerbation rate (RTE-R). In this study, data from an unselected pediatric cohort of CF patients monitored at our tertiary care CF center was used to study the association between CT and RTE-R. From all CT scores, bronchiectasis score was the strongest predictor, with no significant added effect of the other CT scores. In addition, the bronchiectasis score was found to add significantly to the predictive value of FEV₁. The association between bronchiectasis and RTEs found in our study confirms the results of previous studies in young CF patients with mild disease enrolled in a clinical trial (12), in non-CF bronchiectasis patients (13), and in patients with chronic obstructive pulmonary disease.(14)

The association between CT and RTE-R is an important step in the validation process of CT as surrogate endpoint, as RTE-R is considered a clinical important endpoint. A potential limitation of our study was its retrospective study design. However, we selected a robust definition of RTEs, which were defined as the need for intravenous antibiotic treatment for pulmonary deterioration and increased symptoms. In CF, there is no accepted consensus on the definition of an RTE. Our definition has been used in other studies. (9, 24-25) Using this definition, RTEs were unlikely to be missed. Another limitation was that our cohort consisted of mainly pediatric patients with mild to moderate CF lung disease. Whether a similar correlation between bronchiectasis and RTE-R exists in infants and young children with early disease or in the adult population with more advanced lung disease needs to be further investigated.

The sixth important finding of our studies is that the SALD infection/inflammation CT score is significantly associated with survival. In this study, we used data and CTs from CF patients with SALD screened for lung transplantation (LTX) between 1990 and 2005 from 17 centers worldwide. All scans were scored using a semi-automated approach based on our previously developed SALD CT scoring system.(11) Using data of patients who were subsequently listed for LTX after

screening (n=366), we found a significant association between SALD infection/inflammation score and survival. Many studies have aimed to identify better predictors of survival, but CT parameters have never been included in survival modeling.(15-20) The prognostic value of CT for survival has been shown for idiopathic pulmonary fibrosis (39-40). For this reason CT has recently been incorporated in the transplantation guidelines for these patients.(41) The association between CT and survival is an important finding, as it suggests that CT can be used to improve survival prediction models, such as the lung allocation score (LAS). The LAS was introduced in the USA in May 2005 with the aim to reduce waiting list mortality by prioritizing candidates based on urgency, and thus, “de-emphasizing the role of waiting time and geography.”(21) In practice, this means that LTX candidates are ranked according to their LAS. In our study, we found that both SALD infection/inflammation score and LAS had significant, independent predictive value for survival. The retrospective study design was a limitation in our study. We used data from patients listed for LTX between 1990 and 2005 and calculated their LAS. However, the LAS was introduced in May 2005. Thus, most patients were listed in the pre LAS era. In addition, the criteria to select patients for LTX screening were based on the American Thoracic Society guidelines of 1998 (22), which were updated in 2006.(23) In addition, patient survival has improved over the years. (24-26) Although we found no trend for improved survival with listing year in our study, these factors may affect the generalizability of our results to patients currently awaiting LTX. Prospective studies are needed to further establish the role of CT as a predictor for mortality and LTX outcome.

The association between the SALD infection/inflammation score and waiting list mortality also provides an important missing step in the validation of CT as surrogate endpoint. For FEV₁ the link with mortality has been well established in the eighties (15-20, 27-32), and FEV₁ is still the most extensively validated surrogate endpoint in CF. Our finding that SALD infection/inflammation score is linked with mortality is essential for the validation of CT as surrogate endpoint for CF clinical trials.

Other directions for future research

In addition to the findings and directions for future research based on the findings discussed above, there are a number of other opportunities to improve the role of chest imaging for CF lung disease

Lowering radiation dose

Radiation dose is the major limitation of chest CT as a monitoring tool for CF lung disease. To minimize radiation risk of CT scanning to patients, CT protocols should be performed using doses as low as reasonably achievable. Apart from our suggestion to use only one expiratory chest CT to detect and monitor CF lung disease, there may be a role for improvements in CT technologies and reconstruction algorithms to further reduce radiation dose and thus improve the risk benefit ratio. One such new CT technology is the multi detector CT scanner. This type of scanner was introduced in the nineties and, due to its fast acquisition time, may become the new standard. Some studies have attempted to address the effect of such scanners on radiation dose (47-48), but more studies are needed.

Chest magnetic resonance imaging (MRI) has been suggested as radiation free alternative for chest CT to monitor CF lung disease. The sensitivity of MRI to depict large morphological changes has been estimated to be comparable to CT.(33) However, its sensitivity to detect early and smaller changes in lung structure is considered to be inferior to that of chest CT.(33-35) Clearly, MRI needs to be adequately validated to establish its role in monitoring CF lung disease.

Trapped air

The studies performed in CF patients with SALD clearly demonstrate the importance of TA as important component of end stage CF lung disease. TA reflects a combination of small airways disease and diffusion defects.(20) Recent studies in children with CF diagnosed by newborn screening show that TA is present early in life.(10, 36) This makes TA an important treatment target. However, little is known about the reversibility of TA. There is evidence that dornase alpha can reduce TA in mild to moderate CF lung disease (6). The effect of dornase alpha in CF patients with advanced disease has been studied. However, TA detected by CT was not included as an endpoint.(37) In infants, lower CT scores for TA were reported after dornase alpha treatment, however, similar changes were found after placebo treatment and the significance of these changes was not reported.(50) Thus, more studies on the effect of dornase alpha on TA are needed.

For TA detection, both PFT and CT can be used. To our knowledge, there is no gold standard to determine TA, and how PFT estimates of TA relate to CT estimates of TA is not clear. Further longitudinal studies comparing both modalities are needed to identify the optimal strategy to monitor TA. In addition, the optimal CT technique should be assessed. Nowadays, CT inflation level can be standardized using controlled-volume techniques. It has been shown that the level of inflation affects the volume of TA visible on CT.(38) Future studies aimed to quantify the effect of these techniques on the assessment of TA are needed.

CT standardization

Our studies underline the potential use of chest CT in clinical management and clinical studies. However, the use of chest CT in clinical trials requires further standardization of CT protocols. This is especially important in multi-center studies. Protocol standardization will avoid bias related to differences in image resolution and improve the sensitivity and reproducibility of the CT parameters quantified by CT scoring or automated image analysis techniques. The type of protocol will depend on the aims of the study. Standardization of the level of inflation is an important condition that can be achieved using a spirometer or pressure-controlled ventilation techniques. Standardization is especially important for automated CT quantification.

Image analysis

In our studies, we primarily used manual and semi-automated scoring systems. To date, there are no fully automated image analysis systems available that can generate clinical relevant numbers in CF lung disease. Fully automated systems have the advantage of allowing more rapid analyses with near perfect intratest reproducibility. Various systems have been developed that can measure airway wall thickness and bronchial internal diameter (8, 39-45), and TA in CF.(38, 43, 46) However, further cross-sectional as well as longitudinal studies are needed to further refine and validate these systems for use in clinical care and studies.

Further validation of CT

Overall we strongly feel that chest CT can supply highly relevant information. However, the role of chest CT as an accurate and sensitive monitoring instrument for CF lung disease can be further strengthened. An important next step in the validation of CT as a surrogate endpoint is to determine how CT findings impact clinical management. In addition, treatment algorithms based on the CT findings are needed. Furthermore, it is important to establish which change in CT score is clinically relevant. Thus, more comparative studies that include CT scores as well as a traditional clinical endpoint are needed. In addition, more longitudinal studies will be necessary to establish the trends of the different CT features over a defined period of time. This will help defining the required time span for intervention studies.

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Chapter 10

Summary

Chapter 1 contains a general introduction to cystic fibrosis (CF), and states the aims of the studies performed in this thesis

In the first section of the thesis we describe the studies that aimed to optimize computed tomography (CT) protocols in CF. One major disadvantage associated with the use of CT is that it exposes patients to ionizing radiation. Therefore, radiation dose should be as low as reasonably achievable. In CF, a CT examination usually consists of an inspiratory scan to assess structural changes and an expiratory scan to assess trapped air. If expiratory scans can be used to assess structural abnormalities as well, CT examinations would be limited to a single scan. This could reduce radiation dose substantially. We investigated whether this may be possible in **chapter 2**. In this study, we compared low dose inspiratory and ultra low dose expiratory CT scores to determine whether expiratory CT alone may suffice for monitoring the structural changes in CF lung disease. In- and expiratory CTs of 20 children were scored using the Brody-II CT scoring system to assess bronchiectasis, airway wall thickening, mucus plugging, and opacities. We found that CT scores of both scans matched closely, suggesting that ultra low dose expiratory scans alone may be sufficient for monitoring CF lung disease. This would reduce radiation dose for a single investigation by up to 75%.

Trapped air (TA) is an important early change in CF lung disease, and can be assessed using pulmonary function tests (PFTs) or CT. However, little is known about the relationship between these two modalities. In addition, the course of TA over time has not been well studied. For the quantification of TA on CT, it is common practice to use only 3 expiratory slices. However, it is unclear whether this approach is sensitive enough, since the effect of the number of slices on the assessment of TA has never been evaluated in CF. This was the subject of our study described in **chapter 3**. In addition, we compared CT and PFT estimates of TA cross-sectionally and longitudinally. Twenty children contributed 2 routine expiratory CTs and two PFTs over two years. From the volumetric follow up CT, we composed 7 sets with a decreasing number of slices. The last two sets contained 5 and 3 slices. Longitudinal follow-up was done with 3 slices. Trapped air on CT was scored using the Brody-II scoring system and a newly developed quantitative scoring system. We concluded that in general, there was good agreement between TA scores of set 1 (volumetric CT) and set 2 to 7 (CTs with a spacing of respectively 2.4, 4.8, 9.6, and 20.4 mm, and 5 and 3-slice CTs) for both scoring systems (all ICC>0.75). However, the number of CT slices affected the TA assessment using Brody-II scores; mean scores from 5- and 3-slice sets were respectively 7% and 10% lower than mean scores from the volumetric set ($p=0.01$ and $p<0.001$). This suggests that commonly used 3-slice protocols underestimate TA in CF using the Brody-II system. Furthermore, CT and PFT estimates were not correlated, and showed no change over time.

In the second section of the thesis we describe the studies that aimed to further improve image analysis of CT scans of CF patients with severe advanced lung disease (SALD). To quantify the abnormalities on these CTs, we developed a new quantitative CT scoring system for SALD in **chapter 4**. Using this system, we determined the spectrum of structural abnormalities on CT. In this study, 57 CT scans from CF patients screened for LTX in 3 centers were used to design the SALD scoring system. Lung tissue was divided into 4 components: infection/inflammation (including bronchiectasis, airway wall thickening, mucus and consolidations) air trapping/hypoperfusion, bulla/cysts, and normal/hyperperfused tissue. We investigated the correlation between the SALD scoring system and the Brody-II scoring system. With the SALD system, we were able to identify a wide spectrum of structural abnormalities ranging from predominantly infection/inflammation to predominantly air trapping/hypoperfusion. This spectrum may have implications for the clinical management of CF patients with SALD. Furthermore, infection/inflammation scores correlated with Brody-II scores.

In the third section of the thesis we describe the studies that aimed to validate CT as surrogate endpoint in CF. Clinical trials are important to test and optimize new therapeutic interventions. To evaluate the effect of these interventions, sensitive outcome measures are needed. CT holds great potential for use as surrogate endpoint, but needs to be validated for this purpose. In the validation process, it is important to know the course of CT abnormalities over time.

For TA, we previously investigate the course over time, however, we were limited to only 3 expiratory CT slices which was probably not sufficient for sensitive monitoring. In **chapter 5**, we used volumetric CT scans to investigate the changes in TA volume and distribution over 2 years time using automated image analysis software. Localized changes in TA were assessed by matching the follow up CT on the baseline CT, and measuring the volume of stable TA (TA_{stable}), disappeared TA ($TA_{\text{disappeared}}$) and new TA (TA_{new}). The proportion of TA_{new} and $TA_{\text{disappeared}}$ were found to be significantly higher than TA_{stable} . Visual assessment suggested that only TA_{stable} was accurately assessed. We concluded that TA has a stable component over time, reflecting irreversible peripheral airway damage. In addition, TA on CT was not progressive over 2 years.

Another important step in the validation process is to show that CT scores are associated with important clinical outcomes, such as respiratory tract exacerbation rate (RTE-R) and survival. In **chapter 6**, we investigated the association between CT scores and RTE-R in a cohort of pediatric CF patients. We collected CT scans and PFTs made during annual check up when clinically stable, and tracked the number of RTEs in the two years after the CT. We found that bronchiectasis score and FEV1 were both strong independent predictors of RTE-R in the subsequent 2 years. Categorizing the bronchiectasis score in quartiles, RTE-R increased by factors of 1.8, 5.5, and 10.6, respectively.

In **chapter 7** we investigated the association between CT scores and survival, using a semi-automated version of the SALD scoring system. In addition, we studied the value of CT scores to a currently used survival prediction model; the lung allocation score (LAS). We also determined whether the SALD spectrum found in chapter 6 could be reproduced in this larger cohort. For this study, we collected data and CT scans of CF patients with SALD screened for LTX between 1990 and 2005 from 17 centers worldwide. Using data of all patients (n=411), we observed the same SALD spectrum in this cohort as previously observed. Of these patients, 366 entered the waiting list after screening. Using this cohort, we found a significant association between SALD infection/inflammation score and survival, with a hazard ratio per 10 percentage point increase in score of 1.45. The LAS was also significantly associated with survival, with a hazard ratio per point increase in score of 1.1. Furthermore, infection/inflammation score was shown to add significantly to the predictive value of LAS. This study provides an important step in the validation of CT as surrogate endpoint, and strongly suggests that CT scores can add relevant information to survival prediction models such as the LAS.

In **chapter 8**, we reviewed what is known about the use of CT as surrogate endpoint and what research could further strengthen the validation portfolio of CT.

The discussion of the findings of the studies included in this thesis and suggestions for further research are provided in **chapter 9**.

Chapter 11

Samenvatting

Hoofdstuk 1 geeft wat algemene informatie over de ziekte cystische fibrose (CF), en beschrijft het doel van de studies die in dit proefschrift staan.

In hoofdstuk 2 en 3 staan de studies die tot doel hadden om computer tomografie (CT) protocollen van de longen in CF te optimaliseren. Een nadeel van het gebruik van CT is dat het patiënten blootstelt aan straling. Deze straling kan kankerverwekkend zijn. Daarom is het belangrijk dat de stralingsdosering zo laag mogelijk wordt gehouden. Gewoonlijk bestaat het CT protocol voor CF patiënten uit 2 scans; 1 scan na diep inademen en 1 scan na diep uitademen. De inademing scan wordt gebruikt voor het beoordelen van afwijkingen aan de grote luchtwegen. De uitademing scan wordt gebruikt voor het beoordelen van schade aan de kleine luchtwegen. Op CT scans is deze schade zichtbaar als 'trapped air' (TA). Het is onbekend of de uitademing scan alleen voldoende zou kunnen zijn om alle belangrijke afwijkingen te beoordelen. Als dit zo is, dan kan de inademing scan voortaan uit het protocol weggelaten worden. Dit zou de hoeveelheid straling drastisch kunnen verminderen. Of dit mogelijk is, is onderzocht in **hoofdstuk 2**. In deze studie werd gekeken of CT scores van inademing scans hetzelfde waren als CT scores van uitademing scans. Voor dit onderzoek werden 20 in- en uitademing CT scans van kinderen met CF gebruikt. Alle scans werden gescoord met een CT score systeem (Brody-II). Dit was nodig om de ernst van de long afwijkingen om te zetten in een getal. Met het Brody-II systeem werden scores gegeven aan afwijkingen van de luchtwegen (bronchiectasiën), luchtwegwand verdikking, slijm pluggen, en andere afwijkingen. De inademing scan werd gemaakt met een lage stralingsdosering, en de uitademing scan werd gemaakt met een extra lage stralingsdosering. De resultaten lieten zien dat de CT scores van beide scans goed overeen kwamen. Dit suggereert dat een uitademing scan alleen genoeg zou kunnen zijn voor het beoordelen van long afwijkingen bij CF. Dit zou de stralingsdosis voor een enkel CT onderzoek van de longen kunnen verminderen met 75%.

TA is een belangrijke en vroege afwijking in CF longziekte, en ontstaat door schade aan de kleine luchtwegen. Om afwijkingen aan de kleine luchtwegen op te sporen kan naast CT ook gebruik worden gemaakt van longfunctie testen. Er is maar weinig bekend over de relatie tussen TA op CT en de longfunctie testen gevoelig voor de kleine luchtwegen. Verder is het onduidelijk hoe TA verloopt over de tijd. Om de ernst van TA met CT te bepalen worden vaak maar 3 plakjes van de longen gemaakt. Er is nooit onderzocht of deze aanpak wel gevoelig genoeg is om de ernst van TA goed in te schatten. Dit was het onderwerp van **hoofdstuk 3**. Ook zijn CT en longfunctie waardes gevoelig voor de kleine luchtwegen met elkaar vergeleken in deze studie. Hiervoor is gebruik gemaakt van een groep van 20 kinderen met CF die allemaal 2 uitademing CT scans en longfunctie testen over twee jaar tijd hadden gehad. Van de CT zijn 7 sets met een afnemend aantal CT plakjes samengesteld. De laatste twee sets bevatten slechts 5 en 3 plakjes. Om te onderzoeken hoe TA verloopt over de tijd werd gebruik gemaakt van 3 plakjes. TA op CT werd

gescoord met het Brody-II score systeem en een nieuw ontwikkeld, deels gecomputeriseerd score systeem. Deze studie liet zien dat TA scores van set 1 (alle plakjes) en CT scores van set 2 tot en met 7 goed overeen kwamen. Dit gold voor beide score systemen. Echter, de TA bepaling was minder nauwkeurig bij gebruik van 5 of 3 plakjes. Dit doet vermoeden dat de veelgebruikte CT protocollen met maar 3 plakjes de hoeveelheid TA minder nauwkeurig weergeven. Er werd geen verband gevonden tussen CT en longfunctie waardes voor TA, en over de tijd lieten ze geen verandering zien.

In **hoofdstuk 4** staat de resultaten van de studie die tot doel had om de beeldanalyse van CT scans van CF patiënten met vergevorderde long ziekte (SALD) te verbeteren. Om de afwijkingen op deze CT scans te kunnen meten is een nieuw CT score systeem voor SALD ontworpen. Voor deze studie is gebruik gemaakt van 57 CT scans van CF patiënten met ernstige longziekte die gescreend waren voor longtransplantatie (LTX). Al het longweefsel op de scans werd ingedeeld in 1 van de volgende 4 categorieën: infectie/inflammatie (bevat bronchiectasiën, luchtwegwand verdikking, slijm pluggen en consolidaties) air trapping/hypoperfusie, bullae/cysten, en weefsel met een normale/hyperperfusie. Daarnaast werden alle scans ook gescoord met het Brody-II score systeem. Daardoor kon de relatie tussen het SALD score systeem en het Brody-II score systeem worden onderzocht. De resultaten lieten een sterke relatie zien tussen de SALD infectie/inflammatie score en de Brody-II scores. Bovendien werd er een spectrum van afwijkingen bij patiënten met SALD gevonden. Dit spectrum varieerde van voornamelijk infectie/inflammatie-gerelateerde afwijkingen tot voornamelijk air trapping/hypoperfusie-gerelateerde afwijkingen. Wij denken dat deze bevinding belangrijk is voor de behandeling van CF patiënten met SALD.

In hoofdstuk 5 t/m 7 staan de resultaten van de studies die tot doel hadden om CT verder te valideren als uitkomstmaat in CF. Klinische studies zijn nodig om nieuwe medicijnen bij CF patiënten te testen. Om het effect van deze nieuwe medicijnen te kunnen beoordelen zijn gevoelige uitkomstmaten nodig. CT kan een belangrijke uitkomstmaat zijn, maar moet eerst uitgebreid getest worden met validatie studies. In het validatie proces is het belangrijk om het verloop van CT afwijkingen over de tijd te meten. Dit was het onderwerp van **hoofdstuk 5**. In deze studie zijn CT scans gebruikt die de hele long nauwkeurig weergeven. Deze scans bestaan vaak uit 200 plakjes of meer. Met deze scans is gekeken naar de veranderingen in het volume van TA en de verdeling over 2 jaar tijd. TA werd gemeten met nieuw ontwikkelde automatische beeldanalyse software. Om de veranderingen van TA over de tijd te beoordelen werden 'follow up' CTs bovenop de eerste CT gelegd. Zo konden de verschillen in TA volume en verdeling over de tijd goed zichtbaar gemaakt worden. De conclusie van deze studie was dat ongeveer een derde van de hoeveelheid TA stabiel is over de tijd. Dit wijst op onherstelbare schade aan de kleine luchtwegen. Hoopgevend is dat een groot deel van TA nog wel veranderlijk lijkt te zijn en

dus mogelijk gevoelig is voor behandeling. Met deze gevoelige methode werd geen toename van TA gezien over 2 jaar tijd.

Een andere belangrijke stap in het validatie proces is het aantonen dat CT scores een relatie hebben met belangrijke klinische uitkomsten, zoals het aantal ziekenhuisopnames en overleving. In **hoofdstuk 6** is de relatie tussen CT scores en ziekenhuisopnames onderzocht in een groep kinderen met CF. Hierbij werd gebruik gemaakt van CT scans en longfunctie testen gemaakt tijdens de jaarlijkse uitgebreide polikliniek controle van de patiënten. Er werden alleen gegevens verzameld van patiënten die op dat moment niet acuut ziek waren. Ook werd het aantal ziekenhuisopnames in de twee jaar na de CT voor alle patiënten uitgezocht. De resultaten lieten zien dat zowel de CT score voor bronchiectasiën als de longfunctiemaat FEV₁ zeer sterke en onafhankelijke voorspellers waren voor het aantal ziekenhuisopnames in de 2 jaar na CT.

De relatie tussen CT scores en overleving is onderzocht in **hoofdstuk 7**. De CT scans in deze studie werden gescoord met half geautomatiseerde versie van het SALD score systeem. Daarnaast is gekeken naar de toegevoegde waarde van CT scores aan een veel gebruikt model om overleving te voorspellen. Dit model heet de long allocatie score (LAS). Bovendien is gekeken of in deze grote groep patiënten hetzelfde SALD spectrum aanwezig was als eerder beschreven in hoofdstuk 6. Voor deze studie zijn gegevens en CT scans verzameld van CF patiënten met SALD die tussen 1990 en 2005 gescreend waren voor LTX. Aan dit onderzoek werd meegewerkt door 17 transplantatie centra wereldwijd. Gebruikmakend van de gegevens van 411 patiënten is hetzelfde SALD spectrum in deze patiëntengroep gevonden als beschreven in hoofdstuk 6. Van de 411 patiënten kwamen er 366 vervolgens op de wachtlijst voor LTX na de screening. In deze groep werd een duidelijke relatie gevonden tussen de SALD infectie/inflammatie score en overleving. Ook de LAS had een duidelijke relatie met overleving. Bovendien werd aangetoond dat de SALD infectie/inflammatie score een bijdrage kon leveren aan de voorspellende waarde van de LAS. De uitkomsten van deze studie zijn zeer belangrijk voor het validatie proces van CT als uitkomstmaat in CF. Bovendien suggereren deze resultaten heel sterk dat CT scores belangrijke informatie toevoegen aan bestaande voorspellingsmodellen voor overleving zoals de LAS.

In **hoofdstuk 8** is samengevat wat er bekend is over het gebruik van CT als uitkomstmaat in CF. Ook wordt beschreven wat voor onderzoek er verder nog nodig is voor de validatie van CT. De discussie van de resultaten van de studies in dit proefschrift en de suggesties voor verder onderzoek zijn gegeven in **hoofdstuk 9**.

Chapter 12

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Hopital Cochin, Paris; Dr. I. Sermet, thank you for enabling me to collect data at your center. Dr. R. Kanaan, dear Reem, thank you for preparing the data for me so it was easy to find during the visit, and for lending me your office while being away on holiday. It was too bad we didn't have more time to spend than that 1 hour just before you left. I wish you all the best with your family.

Sick Children's Hospital, Toronto; Dr. M. Solomon, dear Melinda, thank you for making it possible for me to squeeze in a last visit before maternity leave, and for sending me the CTs afterwards. Dr. H. Grasemann, thank you for enabling me to

collect data at your center. I would also like to thank Victoria Schnell for replying to all my emails and to arrange all the practical details which enabled me to visit your center at very short notice.

Ondanks dat ik veel op reis ben geweest, was de poli kinderlongziekten een soort van ‘veilige thuishaven’. Daar wil ik mijn directe collega’s Daan, Marije, Esther, Leonie en Sandra bedanken voor de gezelligheid en het meedenken met de praktische vragen die er tijdens het onderzoek opkwamen. Els en Evelien, bedankt voor alle kleine doe-dingetjes en ik hoop dat jullie de traditie van gezellige kameretentjes in stand kunnen houden! Ook de rest van het pulmoteam zou ik graag willen bedanken voor het meedenken naar oplossingen tijdens de research besprekingen voor alle problemen waar ik tijdens mijn onderzoek tegenaan liep. Niet op de poli, maar minstens zo belangrijk is Irma Stok-Beckers, bedankt voor alle praktische ondersteuning en het maken van de vele afspraken met “de baas”, enne, wat mij betreft is bewezen dat Irma (bijna) alles weet! Daarnaast wil ik ook Wim Hop enorm bedanken voor het beantwoorden van alle statistische vragen en het nalezen van mijn manuscripten met betrekking tot de statistiek. Ook de analyses in het artikel over CF exacerbaties en het survival artikel zijn grotendeels van jouw hand. Heel erg bedankt hiervoor en ik hoop dat je wat rust krijgt als je straks met pensioen bent en van het Franse leven kan genieten.

Verder wil ik graag mijn mede-auteurs bedanken; Marcel van Straten, beste Marcel, bedankt voor je snelle en nauwkeurige antwoorden op mijn vragen op CT gebied. Het was altijd gezellig om met jou overleg te hebben. Krista Gerbrands, beste Krista, bedankt voor het scoren van de CTs van de exacerbatie studie. Een hele klus die je toch maar mooi gedaan hebt. Ik wens je veel succes bij je opleiding tot radioloog. Dr. P. A. de Jong, beste Pim, bedankt voor het introduceren van mij bij harm en daarmee het begin van onze samenwerking. Het is een mooi project geworden waar ik met een goed gevoel op terug kijk. Ook jij hebt gescoord voor 1 van mijn studies, heel erg bedankt hiervoor. Ik wens je veel succes in je verdere carrière als radioloog en thuis bij de kindjes. Dr. M. Rosenfeld, dear Margaret, thank you so much for your critical reading a few of my manuscripts. It was always a pleasure to find your input in such a friendly way. It was a pleasure to meet you at the NACF conferences a couple of times. I wish you all the best.

Ook de lunchcollega’s wil ik graag bedanken voor de lunches de afgelopen jaren. Ik wens jullie heel veel succes met jullie studies en zie jullie proefschriften graag tegemoet!

Onderzoek vergt niet alleen veel tijd op het werk, de grens tussen werktijd en privé tijd is regelmatig vervaagd. Ik wil graag mijn vrienden bedanken die er altijd voor me zijn geweest. Lieve Anja, (+Hans en Juliette), ik ben blij dat we ondanks de afstand onze vriendschap in stand hebben weten te houden en verheug me op nog

heel veel etentjes in Utrecht. Lieve Eva (+Arnold), met jou kon ik goed relativeren over het (werkende) leven als dingen me soms boven het hoofd groeide. Ik hoop dat we elkaar nog heel lang blijven zien!

Lieve Leny (+Diederik en de kinderen), bedankt voor alle hulp in praktische zin, of het nou laminaat leggen was of het lenen van kinderkleertjes, fijn om vrienden zoals jullie te hebben. Heel veel succes met jullie huisartsenpraktijk! Lieve Suzanne (+Ivo en Eva), samen hebben we veel meegemaakt, vakanties, weekendjes weg en eindeloze lachbuien om dingen die alleen wij begrepen. Ik hoop dat jij, Ivo en Eva ergens in de tijd weer wat dichterbij ons komen te wonen. Lieve Marie Josee (+Murat), ook wij lopen al jaren met elkaar mee en als mede "Doctor" wist jij precies waar ik soms mee zat. Ook privé lijken we (in sommige opzichten ;)) erg op elkaar. Ik hoop nog heel lang de leuke (en minder leuke) dingen met je te kunnen delen, en ik vind het heel erg fijn dat je als paranimf straks naast me staat.

Tenslotte wil ik ook mijn familie bedanken, lieve mama en papa, bedankt voor alle steun and alle mogelijkheden die jullie me altijd gegeven hebben om mijn vleugels uit te slaan. Jullie hebben me altijd gestimuleerd om datgene te doen wat ik echt graag wilde, ook als dat betekende dat ik (tijdelijk) ver weg was in het buitenland. Jullie positieve invloed op mijn reisdrang hebben er ook voor gezorgd dat ik dit project met beide handen heb aangepakt. Op jullie eigen manier voel ik me heel erg gesteund in mijn leven.

Janneke, Niels, Anne en mini#2, ook jullie hebben me altijd gesteund, tijdens verhuizingen, live events en tijdens mijn onderzoek. Bezoeken aan jullie waren voor mij een fijne manier om te kunnen ontspannen, en ik ben blij met zo'n grote zus zoals jij...Fijn dat je mij tijdens mijn promotie gaat ondersteunen als paranimf. Twee (en een half) weten meer dan 1..

Familie Reinstra; Heit en Mem, Albert en Marga met Minke en Eefje, Henriette en Erik met Ilse, Maaïke en Karsten, Esther en Danny: alle verjaardagen met de hele familie waren altijd een fijne afleiding van het serieuze werkleven. Jullie hebben altijd met heel veel belangstelling meegeleefd met hoe het met mij en het onderzoek verliep. Ik vind het fijn om bij zo'n warme en liefdevolle familie te mogen horen.

En tenslotte diegene die het dichtst bij me staan, mijn lief Remco en onze mooie kinderen Sophie en Max. Lieve kanjers, wat is het leven ontzettend mooi met jullie aan mijn zij...Lieve grote schat, ik ben nog steeds zo blij dat ik je "gevonden" heb. Ik had deze onderzoekstijd nooit zo goed kunnen volhouden en volbrengen zonder jouw onvoorwaardelijke steun, het meedenken met allerlei praktische zaken en het enorme vertrouwen in mij. Ik hou ontzettend veel van je en ik laat je nooit meer gaan! Lieve kleintjes, jullie glimlachjes maken me helemaal warm. Ik kijk ernaar uit om jullie groter te zien groeien en voel me enorm trots dat ik jullie mama ben.

Chapter 13

Curriculum Vitae

Martine Loeve was born on March 4th 1980 in Rotterdam, and raised in Zuidlaren and Utrecht. She graduated in 1998 from secondary school at Sint Bonifatius College in Utrecht, and in the same year, she started her medical training at the Erasmus University in Rotterdam.

As a student, she organized the annual study trip for medical students to Florence in 2001. In the same year, she mentored a group of 1st year medical students. During her studies, she has worked in several medical student teams at the Sint Franciscus Gasthuis in Rotterdam. In one of those teams, she assisted midwives and nurses at the delivery ward with their tasks during delivery and provided the first care of the neonate after birth. In 2002, she was selected for an international exchange program of the Erasmus University and spent 5 months performing research at the University of Maryland, School of Medicine in Baltimore, USA.

After obtaining her master degree in 2002 and before the start of her clinical rotations in 2003, she was enrolled in a work & travel program in Australia and New Zealand. During her clinical rotations, she assisted in the data collection of the Generation R birth cohort study by performing interviews with parents on psychiatric well being prior to childbirth. To be able to conduct these interviews, she was trained at the University of Amsterdam (UvA) in using the Composite International Diagnostic Interview (CIDI), a standardized questionnaire. Her (semi) final rotation was practical obstetrics, for which she worked for three weeks under supervision of midwives from a practice in Amsterdam, in affiliation with the University of Amsterdam (UvA). Her final rotation of 3 months was spent at the pediatric department of the Maasstad Hospital in Rotterdam. She obtained her medical degree in 2005 and continued working at the Maasstad Hospital as a pediatric intern. In April 2006, she started as a PhD student at the Erasmus MC Sophia Children's Hospital working on the studies described in this thesis. She lives in Haarlem with Ir. Remco Reinstra and their children Sophie (September 26th 2009) and Max (November 11th 2010).

Chapter 14

List of publications

1. **Loeve M**, van Hal PThW, Robinson P, de Jong PA, Lequin MH, Hop WCJ, Williams TJ, Nossent GD, Tiddens HAWM. The spectrum of structural abnormalities on CT scans from CF patients with severe advanced lung disease.
Thorax. 2009 Oct;64(10):876-82.
2. van der Giessen LJ , **Loeve M**, de Jongste JC, Hop WCJ, Tiddens HAWM. Nocturnal cough in children with stable cystic fibrosis.
Pediatr Pulmonol. 2009 Sep;44(9):859-65.
3. **Loeve M**, Lequin MH, de Bruijne M, Hartmann ICJ, Hop WCJ, Tiddens HAWM. Volumetric ultra low dose expiratory chest CT scans; sufficient to monitor CF lung disease?
Radiology. 2009 Oct;253(1):223-9
4. Gorbunova V, Sporning J, Lo P, Dirksen A, **Loeve M**, Tiddens HAWM, Nielsen M, de Bruijne M. Mass Preserving Image Registration for Lung CT. Submitted
5. **Loeve M**, Gerbrands K, Hop WCJ, Rosenfeld M, Hartmann ICJ, Tiddens HAWM. Bronchiectasis and pulmonary exacerbations in children with CF.
Chest. 2011 Jul;140(1):178-85.
6. **Loeve M**, de Bruijne M, Hartmann ICJ, van Straten M, Hop WCJ, Tiddens HAWM. 3-slice expiratory computed tomography scans are insufficient for monitoring trapped air in CF.
Accepted for publication in Radiology
7. **Loeve M**, Hop WCJ , de Bruijne M, van Hal PThW, Robinson P, Aitken ML, Dodd JD, Tiddens HAWM, on behalf of the Computed Tomography Cystic Fibrosis Survival study group. CT scores are predictive of survival in CF patients awaiting transplantation.
Submitted
8. **Loeve M**, Tiddens HAWM, Gorbunova V, Hop WCJ, de Bruijne M. Reversibility of trapped air on CT scans of patients with cystic fibrosis; an automated approach.
Submitted
9. **Loeve M**, Krestin GP, Rosenfeld M, Brody AS, Stick SM, Tiddens HAWM. Chest computed tomography; a validated surrogate endpoint of cystic fibrosis lung disease?
Submitted

Chapter 15

PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student: Martine Loeve	PhD period: April 2006 – October 2011
Erasmus MC Department:	Promotors:
1. Pediatric Pulmonology	Prof. Dr. H.A.W.M. Tiddens
2. Radiology	Prof. Dr. G.P. Krestin
Research School: Molecular Medicine	

1. PhD training

	Year	Workload (Hours/ECTS)
General academic skills		
- Online HIPAA training course	2007	0.5
- Biomedical English Writing and Communication	2008	4.0
Research skills		
Statistical courses:		
- Introduction to data-analysis	2007	0.7
- Principles of Research in Medicine and Epidemiology	2008	0.7
- Survival Analysis	2009	1.4
In-depth courses		
- ERS School Course “Lung transplantation”	2007	0.7
Seminars/workshops		
- Cystic Fibrosis Young investigators Meeting, Lille (oral presentation)	2007	1
- Radiologendagen, Rotterdam (oral presentation, awarded for best scientific contribution)	2008	0.3
- Nederlands Respiratoir Samenwerkingsverband (poster presentation)	2009	0.3
- Symposium Nederlandse Cystic Fibrosis Stichting (3 poster presentations)	2010	0.3
- Nederlands Respiratoir Samenwerkingsverband (poster presentation)	2010	0.3

International conferences

- 30 th European Cystic Fibrosis Conference, Belek, Turkey (oral & poster presentation)	2007	0.3
- 17 th European Respiratory Society Conference, Stockholm, Sweden (oral presentation)	2007	0.3
- 21 st North American Cystic Fibrosis Conference, Anaheim, USA (oral presentation)	2007	1
- European Society for Pediatric Radiology Conference, Edinburgh, UK (oral & poster presentation)	2008	0.3
- 31 st European Cystic Fibrosis Conference, Prague, Czech Republic (oral presentation)	2008	1
- 18 th European Respiratory Society Conference, Berlin, Germany (oral presentation)	2008	1
- 22 nd Annual North American Cystic Fibrosis Conference, Orlando, USA (5 poster presentations)	2008	1
- SPIE Medical Imaging, Lake Buena Vista, Florida (poster presentation, awarded for best poster)	2009	0.1
- 2 nd World Conference of Thoracic Imaging, Valencia, Spain (oral presentation)	2009	0.3
- 32 nd European Cystic Fibrosis Conference, Brest, France (oral presentation)	2009	0.1
- 23 rd Annual North American Cystic Fibrosis Conference, Minneapolis, USA (poster presentation)	2009	0.1
- 34 th European Cystic Fibrosis Conference, Hamburg, Germany (2 oral poster presentations, awarded with travel grant)	2011	1
- 4 th International Workshop on pulmonary image analysis, Toronto, Canada (oral presentation)	2011	0.2
- 25 th Annual North American Cystic Fibrosis Conference, Anaheim, California (oral & poster presentation)	2011	1

Seminars and workshops

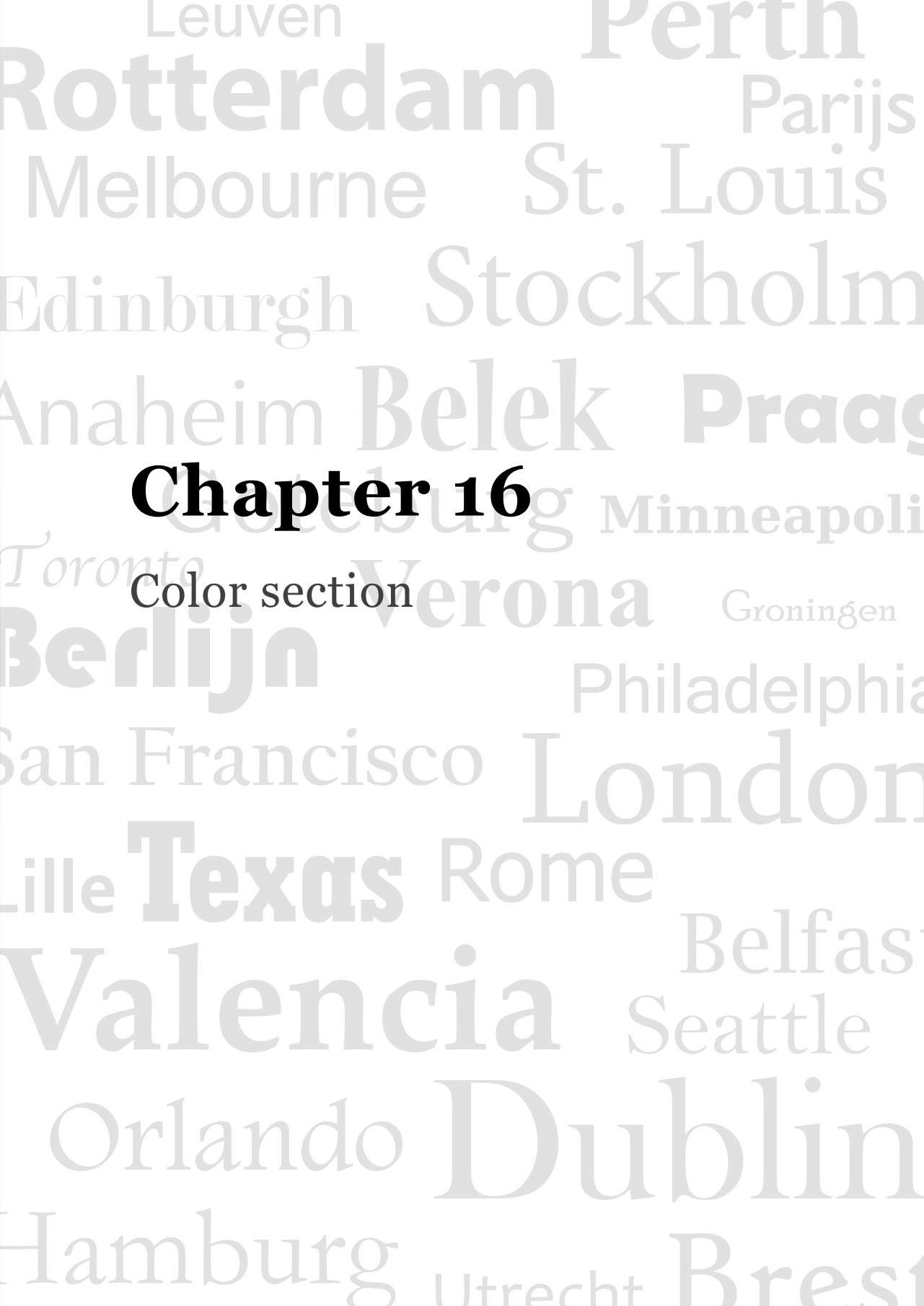
- Presenteren en informatieoverdracht (Postgrade)	14-5-09	0.3
- Workshop Creatief denken (Nederlands Instituut voor Wetenschappelijk onderzoek)	19-5-09	0.3
- Workshop Solliciteren naar een opleidingsplek (KNMG)	15-3-2011	0.2

Didactic skills

- Instructor CT scoring 2006-2010 1

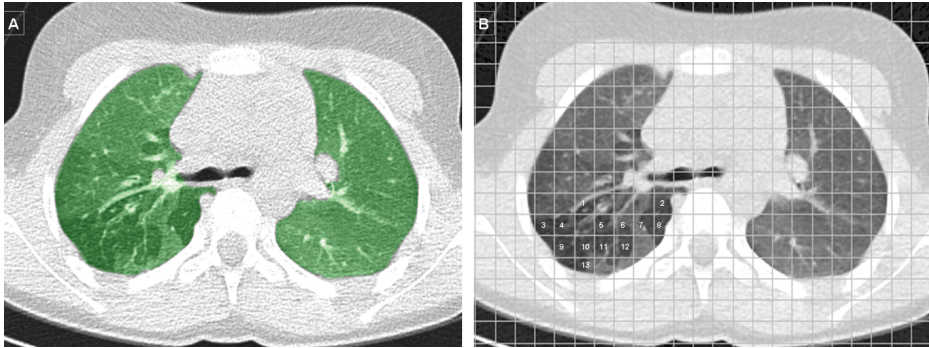
2. Teaching activities

	Year	Workload (Hours/ECTS)
Supervising practicals and excursions		
- Asthma and inhalation medication (1 st year medical students)	2008	0.3
Other		
- Outpatient clinic pediatric pulmonology	2006-2010	6
- Research meeting (every Friday)	2006-2010	3
- Peer review for articles for scientific journals	2008	1.5
Total		30.5

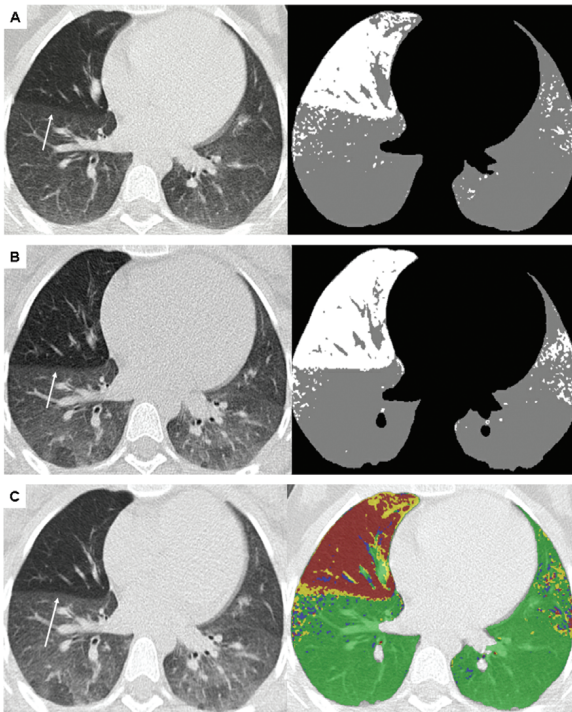


Chapter 16

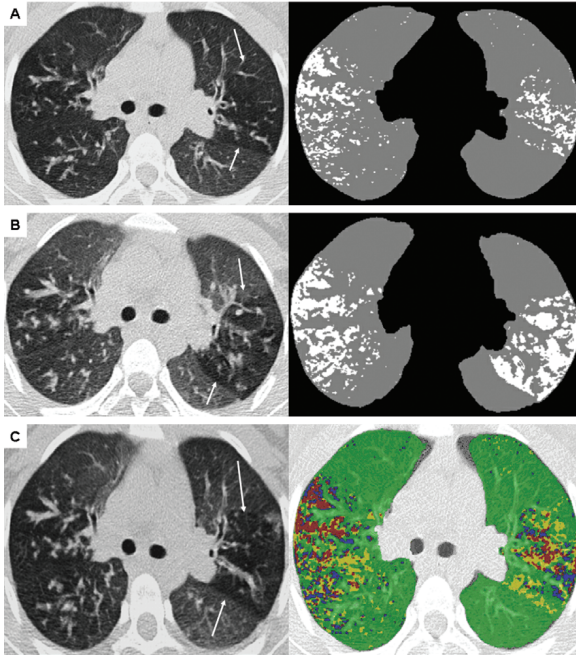
Color section



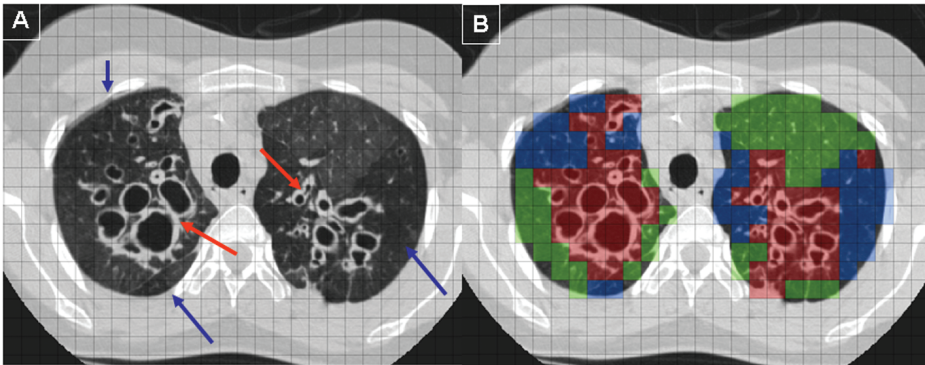
Chapter 3 - Figure 2. CT slices illustrating the new quantitative scoring system. First, the lung volume is automatically segmented (A), and the total lung volume in milliliters is computed. Second, TA volume per slice was assessed using a digital 10x10 mm grid and manually counting the number of cells projected over TA (B). TA volume per slice was then calculated by multiplying the number of TA grid cells by the volume of a grid cell in milliliters. Third, to compute average CT_{TA} volume for the complete scan, TA volumes (in milliliter) of each slice were summed and divided by the total lung volume of the complete scan.



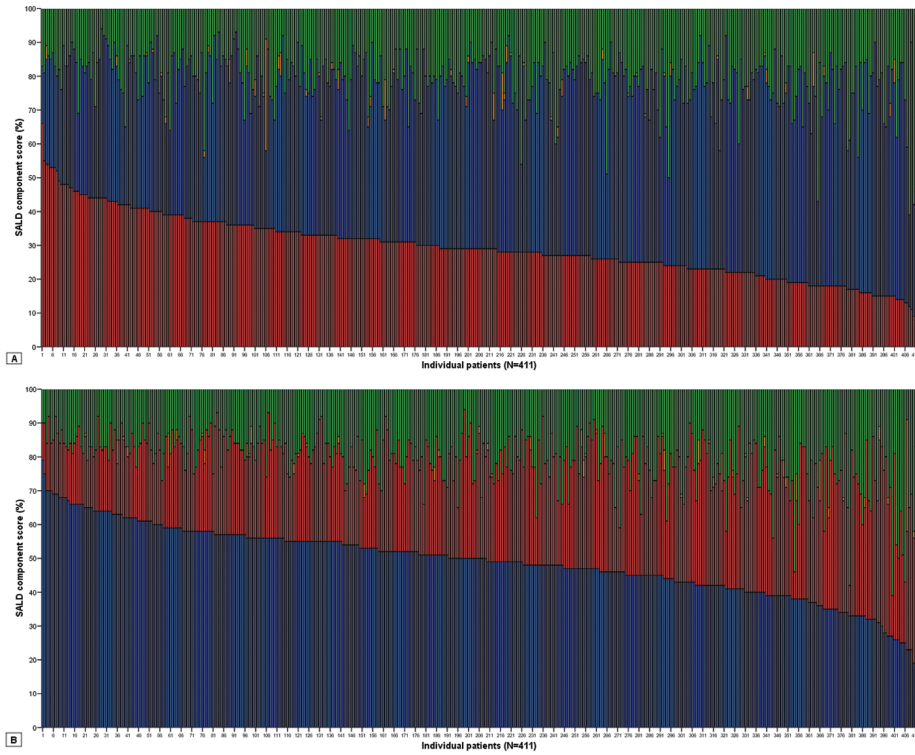
Chapter 5 - Figure 2. Images showing a CT slice of a patient of baseline CT_1 with its corresponding mask showing trapped air (TA, white) and normal lung tissue (grey) (A), follow up CT_2 with its corresponding mask (B), and CT_2 registered to CT_1 with the measured localized TA changes overlaid on CT_1 (C) all taken at the same level. The overlay shows normal lung tissue (green), and the proportion of stable TA (red), new TA (yellow), and reversed TA (blue). Colors highlight only those areas where lung segmentations in CT_1 and registered CT_2 overlap. This figure shows that the large area of stable TA in the right lung (white arrows) is correctly annotated. The relative volume for stable, new, and reversed TA in the slice shown is respectively 20.2%, 6.9%, and 2.7%.



Chapter 5 - Figure 3. Images showing a CT slice of a patient of baseline CT_1 with its corresponding mask showing trapped air (TA, white) and normal lung tissue (grey) (A), follow up CT_2 with its corresponding mask (B), and CT_2 registered to CT_1 with the measured localized TA changes overlaid on CT_1 (C) all taken at the same level. The overlay shows normal lung tissue (green), and the proportion of stable TA (red), new TA (yellow), and reversed TA (blue). Colors highlight only those areas where lung segmentations in CT_1 and registered CT_2 overlap. This figure shows the noise in TA detection. Visually, the area of TA in CT_1 and CT_2 (white arrows) appear quite similar, perhaps with some slight progression. In the segmentation however, these areas were indicated as stable, new, and reversed TA with relative volumes of respectively 5.8%, 10.4%, and 4.5%.



Chapter 7 - Online supplement - Figure E1. Images show a slice at the level of the mid-trachea of a chest CT scan of a CF patient with SALD. Image A shows the original slice on which the abnormalities are indicated by arrows. Red arrows: bronchiectasis, blue arrows: air trapping/hyppoperfusion. Image B shows the same slice after the observer has manually annotated the abnormalities with colours. Red=inflammation, blue=air trapping, green=normal perfused tissue.



Chapter 7 - Figure 2. Visual distribution of the 4 categories of the SALD CT scoring system in the 411 patients who were screened for lung transplant: red = infection/inflammation (INF); blue = air trapping/hyoperfused (AT); green = normal/hyperperfused (NOR); orange = bulla or cysts (BUL). Patients are sorted according to their infection/inflammation component (A) or air trapping/hyoperfusion component (B).

