The Turkish Journal of Pediatrics 2024; 66: 297-308
https://doi.org/10.24953/turkjpediatr.2024.4516

Original Article

The predictive role of lung clearance index on FEV_1 decline in cystic fibrosis

Beste Özsezen[1], Ebru Yalçın[1], Nagehan Emiralioğlu[1], Hande Konşuk Ünlü[2], Dilber Ademhan Tural[1], Canan Caka[3], Birce Sunman[1], Deniz Doğru[1], Ugur Özçelik[1], Nural Kiper[1]

[1]Department of Pediatric Pulmonology, Faculty of Medicine, Hacettepe University, Ankara; [2]Institute of Public Health, Hacettepe University, Ankara; [3]Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Türkiye.

ABSTRACT

Background. The lung clearance index (LCI) is a sensitive lung function index that is used to detect early lung disease changes in children with cystic fibrosis (CF). This study aimed to define the predictive role of baseline LCI, along with other potential factors on the change in forced expiratory volume in one second (FEV_1) during one-year follow-up in CF patients who had a percent predicted (pp) FEV_1 ≥80.

Methods. LCI was concurrently performed on 57 CF patients who had $ppFEV_1$ ≥80 at month zero. The $ppFEV_1$ decline was evaluated prospectively during the one year follow up. The primary outcome of $ppFEV_1$ decline in the study group in one year was dichotomized according to the median value for the decline in $ppFEV_1$, which was 3.7. The LCI value predicting $ppFEV_1$ decline at the end of one year was calculated with receiver operating characteristic curve analysis. Regression analysis was performed. Furthermore, a decision tree was constructed using classification and regression tree methods to better define the potential effect of confounders on the ppFEV1 decline.

Results. The LCI value for predicting $ppFEV_1$ decline >3.7% at the end of one year was 8.2 (area under the curve: 0.80) Multivariable regression analysis showed that the absence of the F508del mutation in at least one allele, LCI >8.2 and initial FEV_1 z-score were predictors of a $ppFEV_1$ decline >3.7 ($p<0.001$). Factors altering $ppFEV_1$ decline>3.7% at the end of one-year evaluated by decision trees were as follows: initial FEV_1 z-score, type of *CFTR* mutation, LCI value and initial weight-for-age z-score.

Conclusions. LCI is sensitive for predicting $ppFEV_1$ decline in patients with $ppFEV_1$ ≥80 along with the initial FEV_1-z-score and type of *CFTR* mutation.

Key words: cystic fibrosis, multiple breath wash-out, lung clearance index, spirometry, FEV_1 decline.

Cystic fibrosis (CF) is one of the most common life-limiting genetic diseases that mainly affects the respiratory system. In recent years, the early diagnosis of the disease, close monitoring of lung health and on-site therapy interventions have prolonged the lives of patients with CF (pwCF). Pulmonary involvement is of early onset and progresses insidiously.[1]

✉ Beste Özsezen
bestekarakaya@hotmail.com

Received 3rd Feb 2023, revised 12th Sep 2023, 6th Dec 2023, 30th Jan 2023, 19th Apr 2023, accepted 10th June 2024.

Conventional spirometry has been the standard test used for evaluating lung function in patients with CF. Failure to perform spirometry effectively in young children and the failure to capture changes in spirometry in the early stages of the disease have led to a search for new tests. The lung clearance index (LCI) derived from the multiple breath washout (MBW) test, is the most commonly used index for measuring ventilation distribution inhomogeneity.[2,3]

The rate of forced expiratory volume in one second (FEV_1) decline is related to disease progression and has been shown to predict

mortality. To date risk factors for FEV_1 decline have been identified as non-modifiable factors such as sex, age, CF transmembrane regulator (*CFTR*) genotype, history of meconium ileus, pancreatic insufficiency, and modifiable factors such as pulmonary exacerbations, airway colonization high initial FEV_1 and CF-related diabetes.[4] However, the predictive role of LCI along with these parameters on FEV_1 decline has not been fully established.

The primary aim of this study was to define the predictive role of baseline LCI derived from the MBW technique, along with other potential factors by decision trees on FEV_1 change in a one year follow up in pwCF who had a percent predicted (pp) FEV_1 ≥80 when LCI was performed. Our hypothesis in this study was that a higher baseline LCI would be related to a higher FEV_1 decline in patients with accompanying factors and we also aimed to investigate these factors.

Materials and Methods

Study design

Children between 5-18 years with a confirmed diagnosis of CF, attending a tertiary pediatric CF clinic, who were able to cooperate to spirometry and LCI and had $ppFEV_1$ ≥80 were enrolled.[5] This was a single-center, prospective cohort study. The study was approved by Hacettepe University Ethics Committee for Non-Interventional Studies (GO 20-637). Patients and caregivers gave written consent to participate in the study. The study was conducted between April 2018- April 2019.

The sample size was calculated using the formula below:

$$n = \frac{Z^2_{1-\alpha/2} \times S_P \times (1 - S_p)}{L^2 \times (1 - Prevalans)}$$

(S_P = anticipated specificity, α = size of the critical region [1 − α is the confidence level], $Z_{1-\alpha/2}$ = standard normal deviation corresponding to the specified size of the critical region [α] L = absolute precision desired on either side [half-width of the confidence interval, CI] of sensitivity or specificity)[6]

According to this formula, when $Z_{1-\alpha/2}$=1.96, SP=0.85, L=0.15 and prevalence=0.39 (based on the Turkish cystic fibrosis registry data, where 39% of the patients have low FEV_1% predicted[7]), the minimum sample size was calculated as 56.

The MBW test was performed at the initial visit at the time of inclusion, along with spirometry. The spirometry was then performed at 3, 6 and 12 months during routine outpatient visits. The study was terminated by performing the last spirometry at the twelfth month after the MBW was performed. The patients' demographic data including weight, height, and body mass index (BMI), transcutaneous oxygen saturation, physical examination findings, microorganism growth and colonization status in sputum culture, were recorded. The radiological examinations findings which were performed according to the decision of the clinician, considering the clinical condition of the patients were recorded. At each visit, patients were questioned for acute pulmonary exacerbation (aPEx) signs and compliance with their standard therapies (medications and chest physiotherapy). All aPEx events and intravenous / oral antibiotic therapies that occurred between visits were recorded.

aPEx was defined according to the study conducted by Fuchs et al.[8] Chronic *P. aeruginosa* infection was defined according to the study by Lee et al.[9] Mucus samples for microbiologic analysis were collected at all test occasions.

Measurements

Patients performed the MBW test using a Nitrogen Analyzer connected to a Sensormedics Vmax Spectra 22 Device. Nitrogen (N_2) gas was used as the inert gas. The MBW tests were performed in alliance with the segregation rules according to the ATS/ERS consensus statement.[10-13] MBW measurements were performed by the same staff using the same equipment. MBW test was performed with

the patient in the sitting position, breathing through a mouthpiece, wearing a nose clip. After a relaxed tidal breath, the patient was switched to 100% oxygen breathing until the end-tidal N_2 concentration declined below $1/40^{th}$ of the starting end-tidal concentration for at least three consecutive breaths. The LCI was calculated as the cumulative expired volume divided by the functional residual capacity (FRC) at 1/40 of the initial N_2 concentration. MBW test completion was established when three technically acceptable tests were achieved. All MBW traces were controlled for technical quality and convenient breathing pattern. Calibration was performed prior to testing on each test day. Quality control of MBW trials were conducted according to the 2013 ATS/ERS MBW consensus guideline.[10-12]

Spirometry was performed according to the ATS/ERS statement.[14] Spirometry completion was established when three technically acceptable tests were achieved. The percent predicted values and z-scores were calculated using all age prediction equations for spirometry from the Global Lung Function Initiative.[15] The decline in $ppFEV_1$ at the 12th month was calculated according to the initial $ppFEV_1$.

Statistical analyses were performed using the SPSS version 25.0 software package (IBM, SPSS, Chicago, IL, USA). Normal distribution of data was tested analytically (Kolmogorov-Smirnov/ShapiroWilk tests) and visually (histogram, probability plots). Categorial variables were described as relative and absolute frequencies. Continuous variables which are normally distributed were summarized as mean ± standard deviation (SD) and analyzed using Student's t-test. Repeated measure ANOVA was used for dependent variables for repeated measures that are normally distributed. For significant ANOVA results, Bonferroni test was used for binary comparisons between groups.

Correlations between parameters were assessed using Pearson correlation coefficient (r). The primary outcome $ppFEV_1$ decline in one year was dichotomized according to the median value for the decline in $ppFEV_1$ in one-year follow-up which was 3.7. To define LCI value to predict $ppFEV_1$ decline at the end of one year the patients were grouped into two: patients who had a decline in $ppFEV_1$ ≤3.7 and patients who had a decline in $ppFEV_1$ >3.7 and it was calculated using receiver operating characteristic (ROC) curve analysis. The cut-off point was determined based on Youden index. Regression analysis was performed to adjust the effect of potential confounders on the $ppFEV_1$ decline. The SMOTE approach was used to rebalance the data. Independent variables that had a relation with dependent variables with a p-value of ≤0.25 in univariable regression analysis were further analyzed in a multivariable regression model. We used backward logistic regression analysis using $ppFEV_1$ decline >3.7 (dichotomous variable) as the dependent variable and presence/absence of F508del mutation in at least one allele, baseline LCI, initial FEV_1 z-score, initial weight for age (WFA) as independent variables.

A decision tree was constructed to better define the potential effect of confounders on the $ppFEV_1$ decline. In the decision tree patients were divided into two groups according to the median $ppFEV_1$ decline >3.7. The decision trees were created using the R programming language version 4.0.0. "Rpart" library was used to construct decision trees. The data was divided into training and test data. During the learning phase, a training dataset was used to develop the classification model. In the second step, the test set was used to evaluate the classification model's accuracy after it had been trained using the training set. Accuracy rate, sensitivity and specificity were measures used to assess the model's performance. The "Performance Estimation" library was used to rebalance data using the SMOTE technique.[16-18]

Results

Over the one-year recruitment period among 360 pwCF aged 0-18 years, 57 subjects with $ppFEV_1$ ≥80 who were able to perform the MBW and

spirometry were enrolled. The demographic, microbiologic, radiologic, treatment data and the latest chest X-rays and thorax CT of the patients are summarized in Table I. During the one-year follow-up, 23 patients (40.3%) had aPEx; 14 (24.6%) patients had one, 8 (14%) patients had two, and one (1.8%) patient had 4 exacerbations. The median pulmonary exacerbation number was 1 (interquartile range [IQR]: 1-2) per patient per year. A total of 54 courses of non-prophylactic antibiotics (oral 84.4%, intravenous 15.6%) were used during the study.

The initial, 3, 6, and 12-month spirometry values and BMI-z-score are summarized in Table II. A significant decline in FEV_1 (pp-z-score) and FEF_{25-75} (pp-z-score) in the third, sixth, and twelfth months was shown by using repeated measure ANOVA. There was no significant decline in BMI z-score at the end of one year.

The mean LCI value was 7.39 (± 2.00). The mean LCI was higher in girls, patients without F508del mutation in at least one allele, patients with pancreatic insufficiency, and patients with bronchiectasis/atelectasis. However, they were not statistically significant (p=0.7, p=0.6, p=0.6, p=0.1 respectively) (Table III). According to the Pearson correlation test, there was no correlation between LCI and initial, and twelfth month $ppFEV_1$ and z-scores (r=-0.1 p=0.46, r=-0.08 p=0.53 for initial $ppFEV_1$ and z-scores; r=-0.18 p=0.20, r=-0.13 p=0.3 for twelfth month $ppFEV_1$ and z-scores). No correlation was found between BMI-z-score and FEV_1, FEF_{25-75} z-scores and LCI.

The median $ppFEV_1$ decline for the study group was 3.7 (IQR: 0.5-6.5). The LCI value for predicting $ppFEV_1$ decline >3.7 at the end of one year was calculated using the ROC curve analysis and was found to be 8.2 (area under the ROC curve: 0.80, sensitivity 46.2% [95% CI: 24.1-68.3], specificity 86.4% [95% CI: 75.7-96.3], p<0.001) (Fig. 1).

Regression analysis was performed to evaluate the effect of covariates on $ppFEV_1$ decline. Age, gender, age at diagnosis, presence/absence of F508del mutation in at least one allele, baseline LCI, initial FEV_1 z-score, total acute pulmonary exacerbations, *Pseudomonas aeruginosa* and *Staphlyococcous aureus* colonization, presence of bronchiectasis ± atelectasis, initial WFA and BMI z-score were selected as covariates for predicting a $ppFEV_1$ decline >3.7 in univariable analysis. Multivariable backward logistic regression analysis with presence/absence of F508del mutation in at least one allele, baseline LCI, initial FEV_1 z-score, initial WFA z-score showed that absence of F508del mutation in at least one allele, LCI >8.2 and initial FEV_1 z-score were predictors of $ppFEV_1$ decline >3.7 (p<0.001, Table IV, accuracy: 0.84, sensitivity: 0.81, specificity: 0.88).

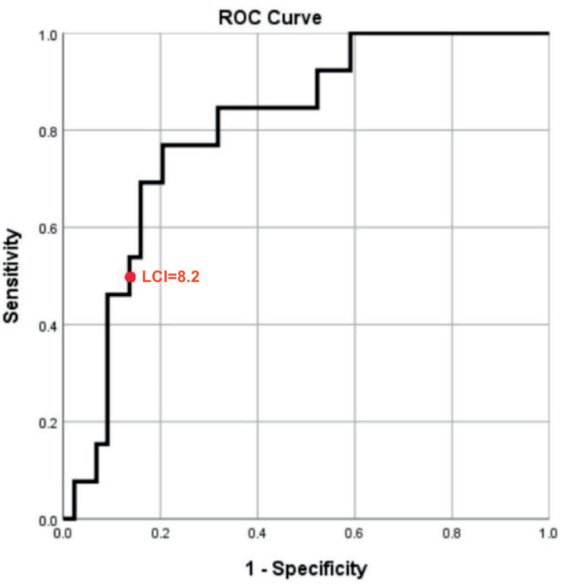

Fig. 1. Receiver operating characteristic (ROC) curve of lung clearance index for predicting ability of FEV_1 decline by > 3.7% at the end of one year. The red dot represents the cut of value lung clearance index=8.2 which is defined by the ROC curve analysis [area under the ROC curve: 0.80, sensitivity 46.2% (95% CI: 24.1-68.3), specificity 86.4% (95% CI: 75.7-96.3), p<0.001]

Table I. Demographic, microbiologic, radiologic, and treatment data of subjects at time of the initial visit (N=57).

Male/female	31/26
Age, yr, mean (SD)	11.6 (3.5)
Age at diagnosis, yr, median (IQR)	0.3 (0.2-1.5)
Genotype, n (%)	
F508del/F508del	10 (17.5)
F508del/other	13 (22.8)
Other/other	34 (59.7)
Exocrine pancreatic insufficiency	50 (87.7)
Chronic liver disease, n (%)	15 (26.3)
Cystic fibrosis related diabetes, n (%)	4 (7)
Weight for age z-score, mean (SD)	-0.31 (1.10)
Height for age z-score, mean (SD)	-0.47 (1.08)
BMI z-score, mean (SD)	0.01 (1.09)
Transcutaneous oxygen saturation (%), mean (SD)	96.9 (1.5)
Microbiological growth (sputum or deep oropharyngeal), n (%)	
Methicillin sensitive *Staphylococcus aureus*	32 (56.1)
Methicillin resistant *Staphylococcus aureus*	9 (15.8)
Pseudomonas aeruginosa	16 (28.1)
Haemophilus influenzae	2 (3.5)
Burkholderia cepacia	2 (3.5)
Stenotrophomonas maltophilia	2 (3.5)
Achromobacter	1 (1.8)
Candida species	9 (15.8)
Aspergillus fumigatus	2 (3.5)
Microbiological colonization, n (%)	
Staphylococcus aureus	42 (71.9)
Pseudomonas aeruginosa	15 (26.3)
Chest X-ray, n (%)	57 (100)
Normal	3 (5.3)
Pulmonary infiltration	3 (5.3)
Chronic changes	51 (86.4)
(air trapping, mosaic pattern, bronchiectasis and/ or atelectasis)	
Thorax CT, n (%)	10 (17.5)
Atelectasis	6 (10.5)
Bronchiectasis	8 (14)
Mucus plugs	8 (14)
Lymphadenopathy	4 (7)
Medications	
Inhaled dornase alpha, n (%)	
Adherent to treatment	52 (91.2)
Not adherent to treatment	3 (5.3)
Not recommended by the physician	2 (3.5)
Inhaled mannitol or hypertonic saline, n (%)*	
At month zero	0 (0)
Chest physiotherapy, n(%)	
Regular	44 (77.2)
Irregular	13 (22.8)

BMI: body mass index, CT: computed tomography, IQR: interquartile range, SD: standard deviation, yr: year.
* Only one patient started inhaled mannitol treatment at the third month, and five patients started inhaled hypertonic saline treatment at the sixth month visit.

Table II. Percent predicted and z-scores of FEV_1 and FEF_{25-75} and BMI z-score at initial, third, sixth and twelfth month.

Spirometry parameters	Clinical visit time (month)				p value
	0	3	6	12	
FEV_1, mean (SD)					
% predicted	107.0 (15.7)	102.6 (17.7)	102.6 (17.7)	101.4 (18.9)	0.001*
z-score	0.55 (1.59)	0.12 (1.90)	0.12 (1.90)	-0.09 (1.98)	0.001**
FEF_{25-75}, mean (SD)					
% predicted	107.2 (35.9)	98.8 (37.4)	98.8 (37.4)	94.1 (37.3)	<0.001+
z-score	0.14 (1.94)	-0.29 (2.11)	-0.29 (2.11)	-0.53 (2.17)	<0.001++
BMI z-score, mean (SD)	0.01 (1.09)	-0.09 (1.06)	-0.09 (1.06)	-0.08 (1.03)	0.5

* FEV_1 %predicted month 0 is statistically significant with FEV_1 %predicted month 6 (sig: 0.01) and FEV_1 %predicted month 12 (sig: 0.01), but not with FEV_1 %predicted month 3 (sig: 0.06). FEV_1 %predicted month 3 is not significant with FEV_1 %predicted month 6 (sig: 0.9) and FEV_1 %predicted month 12 (sig: 0.9). FEV_1 %predicted month 6 is not significant with FEV_1 %predicted month 12 (sig: 0.9).
** FEV_1 z score month 0 is statistically significant with FEV_1 z score month 3 (sig: 0.03) FEV_1 z score month 6 (sig: 0.01), FEV_1 z score month 12 (sig: 0.005). FEV_1 z score month 3 is not significant with FEV_1 z score month 6 (sig: 0.9) and FEV_1 z score month 12 (sig: 0.9). FEV_1 z score month 6 is not significant with FEV_1 z score month 12 (sig: 0.9).
+ FEF_{25-75} %predicted month 0 is statistically significant with FEF_{25-75} %predicted month 3 (sig: 0.01), FEF_{25-75} %predicted month 6 (sig: 0.01) and FEF_{25-75} %predicted month 12 (sig: 0.001). FEF_{25-75} %predicted month 3 is not significant with FEF_{25-75} %predicted month 6 (sig: 0.9) and FEF_{25-75} %predicted month 12 (sig: 0.6). FEF_{25-75} %predicted month 6 is not significant with FEF_{25-75} %predicted month 12 (sig: 0.7).
++ FEF_{25-75} z score month 0 is statistically significant with FEF_{25-75} z score month 3 (sig: 0.02) FEF_{25-75} z score month 6 (sig: 0.01), FEF_{25-75} z score month 12 (sig: 0.001). FEF_{25-75} z score month 3 is not significant with FEF_{25-75} z score month 6 (sig: 0.9) and FEF_{25-75} z score month 12 (sig: 0.9). FEF_{25-75} z score month 6 is not significant with FEF_{25-75} z score month 12 (sig: 0.9).
BMI: body mass index, FEF_{25-75}: forced expiratory flow between 25% and 75% of forced vital capacity, FEV_1: forced expiratory volume in the first second, SD: standard deviation.

Table III. The mean lung clearance index value of patients according to different categories.

	Lung Clearance Index Mean (SD)	p value
Gender (n)		0.7
Female (26)	7.52 (1.40)	
Male (31)	7.28 (1.56)	
CFTR mutation (n)		0.6
F508del in at least one allele present (23)	7.21 (1.74)	
F508del absent (34)	7.51 (2.18)	
Pancreatic insufficiency (n)		0.6
Present (50)	7.46 (1.10)	
Absent (7)	6.88 (0.94)	
Bronchiectasis / atelectasis (n)		0.1
Present (33)	7.76 (2.10)	
Absent (24)	6.90 (1.57)	
Pulmonary exacerbation during one year interval (n)		0.3
Present (23)	7.10 (1.50)	
Absent (34)	7.60 (1.30)	

* *CFTR*: cystic fibrosis transmembrane conductance regulator, SD: standard deviation.

Table IV. Backward logistic regression analysis for variables predicting $ppFEV_1$ decline >3.7.

Model	Odds ratio	95% CI	Sig
Step 1			
Constant	1.34	0.65-2.75	0.4
Absence of F508del mutation	0.014	0.002-0.093	<0.001
LCI >8.2	7.15	2.1-24.0	0.001
Initial FEV1 z- score	0.19	0.09-0.41	<0.001
Initial WFA z -score	1.22	0.62-2.42	0.6
Step 2			
Constant	1.32	0.65-2.70	0.4
Absence of F508del mutation	0.013	0.002-0.082	<0.001
LCI >8.2	7.65	2.31-25.31	<0.001
Initial FEV_1 z- score	0.19	0.09-0.42	<0.001

CI: confidence interval, LCI: lung clearance index, $ppFEV_1$: percent predicted forced expiratory volume in one second, sig: significance, WFA: weight for age.

The independent variables that had a relation with >3.7 $ppFEV_1$ decline in univariate analysis were also analyzed by decision trees. Patients who had $ppFEV_1$ decline >3.7 were grouped as "high", whereas the second group consisted of patients who had $ppFEV_1$ decline ≤3.7 and were grouped as "low" in the decision trees. Only 22.8% of the patients (n:13) had $ppFEV_1$ decline >3.7. To overcome the class imbalance problem in the first step 10% of the dataset was reserved for validation. The SMOTE approach was used to resample and balance the remaining dataset. The data set which became balanced in terms of the class distributions of outcome then were then split into parts: 70% for train data set and 30% for test data set. Using the train data set, a decision tree was created. The first splitting variable is initial FEV_1 z-score followed by *CFTR* mutation analysis, LCI ≤8.2 defined by ROC curve, and initial WFA z-score. The predicted class is shown by colors, with pink representing a decrease of ≤3.7% in FEV_1 and blue representing a decrease of >3.7% in FEV_1.

The classification rules of the decision tree generated by the CART algorithm are given as follows:

1) If a patient's initial FEV_1-z-score is ≥0.62, a decline in $ppFEV_1$ in one year follow up will be ≤3.7 with a 100% probability.

2) If a patient's initial FEV_1-z-score is <0.62 and has a F508del mutation in at least one allele, a decline in $ppFEV_1$ in one year follow up will be ≤3.7 with a 91% probability.

3) If a patient's initial FEV_1-z-score is between -0.60 and 0.62, the patient does not have a F508del mutation, LCI is ≤8.2 and initial WFA-z-score is ≥0.27, a decline in $ppFEV_1$ in one year follow up will be ≤3.7 with an 80% probability.

4) If a patient's initial FEV_1-z-score is between -0.60 and 0.62, the patient doesn't have a F508del mutation, LCI is ≤8.2 and initial WFA-z-score is less than 0.27, a decline in $ppFEV_1$ in one year follow up will be >3.7 with an 88% probability.

5) If a patient's initial FEV_1-z-score is between -0.60 and 0.62, the patient doesn't have a F508del mutation and LCI >8.2, a decline in $ppFEV_1$ in one year follow up will be >3.7 with a 93% probability.

6) If a patient's initial FEV_1-z-score is less than -0.60, a decline in $ppFEV_1$ in one year follow up will be >3.7 (Fig. 2).

The results of the decision tree show that factors altering $ppFEV_1$ decline >3.7% at the end of one year are the initial FEV_1 z-score, type of CFTR mutation, LCI value and initial weight-for-age z-score. According to the results of performance

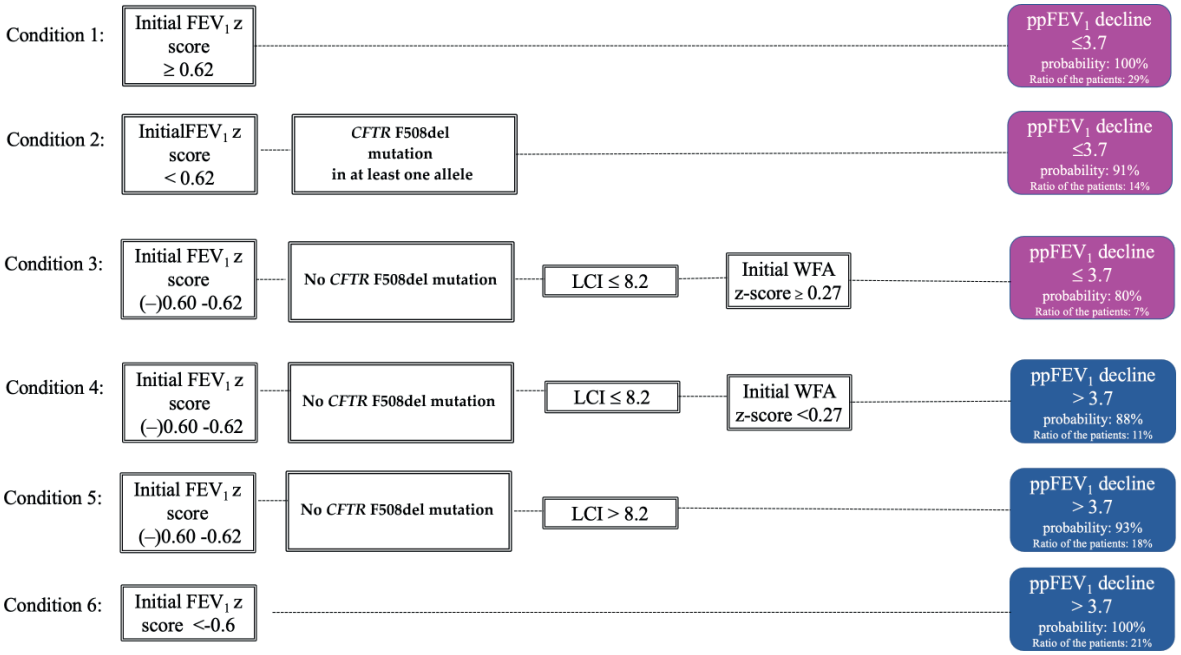

Fig. 2. Decision tree for determining FEV1 decline >3.7.

The first splitting variable is initial FEV_1 z-score followed by CFTR mutation analysis, LCI ≤8.2 defined by ROC curve, and initial WFA z-score. The predicted class is shown by colors, with pink representing a decrease of ≤3.7% in FEV_1 and blue representing a decrease of >3.7% in FEV_1.

The classification rules of the decision tree generated by the CART algorithm are given in the figure and in the text.

*CFTR: cystic fibrosis transmembrane regulator, LCI: lung clearance index, $ppFEV_1$: percent predicted forced expiratory volume in one second, WFA: weight for age.

metrics the model's ability to predict whether a decrease in $ppFEV_1$ in one-year follow-up was lower than or equal to greater than 3.7% which is quite strong (Train dataset accuracy: 94.7%, sensitivity: 95%, specificity 95%; Test dataset accuracy: 87.5%, sensitivity: 81%, specificity: 94%; validation dataset accuracy: 84.6%, sensitivity: 100%, specificity 80%). The detailed results of the decision tree model including the accuracy, sensitivity and specificity and confusion matrix for each dataset (train, test and validation) are shown in Table V and VI.

Discussion

In our tertiary pediatric CF clinic, spirometry was performed on 250 patients during 2019, among these patients 23% had $ppFEV_1$ ≥80. Therefore, this wide cohort of patients required a more sensitive method for monitoring lung health which would enable us to apply the right interventions when necessary. To achieve this goal, we aimed to determine an LCI value that could be a warning for a near-future $ppFEV_1$ decline along with other potential predictors. In this first report of LCI results in pwCF from Turkey designed in a mild CF population including school-age children and adolescents we found that $ppFEV_1$ significantly declined during a one-year follow-up in patients who initially had a $ppFEV_1$ ≥80. This decline in $ppFEV_1$ was >3.7 in almost one quarter of the patients. For this reason, LCI values were calculated using ROC curve analysis; the value that could predict FEV_1 decline >3.7% was found to be 8.2. The other predictors for $ppFEV_1$ decline along with LCI were the initial FEV_1-z-score, type of *CFTR* mutation and initial WFA z-score.

The annual $ppFEV_1$ decline in different studies ranges between 0.06-3.64%.[4] The $ppFEV_1$ decline in our study, which was 3.7%, is significantly higher compared to other studies. The higher

Table V. The accuracy, sensitivity and specificity of the decision tree model.

	Train dataset	Test dataset	Validation dataset
$ppFEV_1$ decline <3.7	38 (50%)	16 (50%)	10 (76.9%)
$ppFEV_1$ decline ≥ 3.7	38 (50%)	16 (50%)	3 (23.1%)
Accuracy	94.74 %	87.5 %	84.62 %
Sensitivity	0.947	0.812	1.000
Specificity	0.947	0.937	0.800
Positive predictive value	0.947	0.928	0.600
Negative predictive value	0.947	0.833	1.000

$ppFEV_1$: percent predicted forced expiratory volume in one second

Table VI. The confusion matrix for train, test and validation dataset for the decision tree model.

Prediction for train dataset, n	Reference for train dataset, n	
	Low	High
Low	36	2
High	2	36
Prediction for test dataset, n	Reference for test dataset, n	
	Low	High
Low	15	3
High	1	13
Prediction for validation dataset, n	Reference for validation dataset, n	
	Low	High
Low	8	0
High	2	3

$ppFEV_1$ decline in our cohort can be explained by the following: First of all, our cohort consisted of children with high baseline $ppFEV_1$ which has been reported as an independent risk factor for FEV_1 decline in several studies.[19-21] The reason for $ppFEV_1$ decline in patients with high baseline FEV_1 can be explained by several reasons. First of all, physicians may underestimate the early decline in FEV_1 which is still within normal limits and not intervene as quickly as possible in pwCF with moderate/severe disease. Secondly, patients might think that being in a state of wellness means there is no need for any treatment and they may not take their standard therapies/physiotherapy either involuntarily (simply forgetting their therapies) or voluntarily (nonadherence). In our study, a quarter of the patients were non-adherent to physiotherapy which we believe is related to the high number of adolescents (n=44) who are prone to being non-adherent to treatments. We believe the second reason for the higher FEV_1 decline when compared with the literature is associated with newborn screening (NBS). It's well known that patients diagnosed with NBS have improved respiratory, nutritional outcomes and survival.[22] NBS for CF was implemented in Türkiye on 01.01.2015 by the Ministry of Health, so none of the patients enrolled in the study were diagnosed through NBS. The third reason for higher FEV_1 decline can be explained by the low rate of inhaled hypertonic saline (HS) or inhaled mannitol which is mainly due to financial obstacles as the drugs were only reimbursed after 2018. All of the factors mentioned above put our mild CF patients at greater risk compared to pwCF with moderate/severe disease.

The mean LCI for healthy populations ranges between 6.2-7.2, and the upper normal limit for LCI, which is defined as mean LCI+2SD, is reported as 7.9-8.2.[23-25] Differences in normal values between studies can be explained by age differences, methods of analysis, software used, devices and set-up, and the tracer gas used. The upper limit of normal (ULN) of LCI for healthy school-age children and adolescents was defined as 7.91.[25] When this upper limit was taken into consideration, almost one quarter of the patients had an elevated LCI. In the study of Ellemunter et al.[26], they found an elevated LCI in almost 80% of the mild pwCF. Similarly, in the 2014 study of Fuchs et al.[24], they showed that 83% had an increased LCI. Also, Fuchs et al.[24] reported a mean baseline LCI of 8. One of the reasons for the lower LCI in the current study may be the difference in FEV_1-z-scores of patients who were enrolled. In our study, the mean FEV_1-z-score was 0.55, whereas it was -0.26 in the study of Fuchs et al. In addition, in the current study, a more homogeneous group by age was included, whereas adults were also involved in Fuchs et al. study.

With the understanding that early lung disease arises in the peripheral airways, LCI has become a tool that can predict future respiratory functions when the patient has preserved spirometry values.[27,28] In our study, the LCI value, predicting near future FEV_1 decline was 8.2 which is slightly higher than the ULN in published reference equations.[25] LCI measurement can be performed in functional lung tissue, therefore it is mostly preferred in mild CF patients in order to detect early lung changes and monitor disease progression. LCI can also be used to detect pulmonary exacerbations and first PA growth.[29,30] Besides, there are several studies evaluating medical treatment (dornase alpha, hypertonic saline, CFTR modulators) responses by LCI measurements.[31,32] Recently, Kurz et al. showed that increased LCI is associated with a greater risk of death or lung transplantation.[33] Future studies are needed to define how to implement LCI monitoring in CF patients in daily practices in terms of LCI monitorization frequency and the change in LCI value. It is also necessary to clearly state whether follow-up with LCI improves management and survival in patients with CF.

Our study has some limitations. This was a single-center study designed without a control group. Compliance with medical therapies was evaluated according to the patients' declaration which may cause information/recall bias. The reported compliance with medical therapies has been shown to be less sensitive and correct by overestimating the real number of performed treatments compared to electronic measures such as inhalers counting the inhalation cycles. Besides the low sensitivity of the LCI cut off defined by the ROC curve to predict FEV_1 decline can be related to the sample size which is within the acceptable limits but still within the lower limit of normal. Also, due to low sensitivity, the false negative rate was high and negative diagnoses were not very reliable. Because of this limitation, our results cannot predict the FEV_1 decline by only LCI, but can predict the FEV_1 decline along with initial FEV_1-z-score, initial WFA z-score and type of *CFTR* mutation. To overcome this limitation larger prospective, longitudinal, multicenter studies are needed. In addition, the relatively small sample size is a problem in terms of applying logistic regression and decision tree models. This problem affects the validity of the results derived from regression and classification models. In addition, since the class distributions are imbalanced, the use of the SMOTE method and the low number of observations in the validation set can reduce the reliability of the results. Last of all, the variability in LCI ranging between 15-25%, which has been shown to be highest in pwCF with more severe lung disease could not be interpreted because we only measured LCI values at the beginning of the study.[34] However, the study conducted by Oude Engberink et al.[35] also showed that the variability is lower in LCI measurements taken 24 hours apart, which suggests that our findings can reflect close to normal LCI values.

Conclusions

Our study confirms that LCI is a helpful tool for predicting FEV_1 decline in patients who have $ppFEV_1 \geq 80$ along with several parameters such as the initial FEV_1 z-score, type of *CFTR* mutation. Given the feasibility of LCI in infants and preschool children, we should encourage its use in children with mild CF, alongside spirometry, to enhance medical treatment strategies and improve survival.

Ethical approval

Informed consent was obtained for participation in the study and the study was approved by Hacettepe University Ethics Committee for Non-Interventional Studies (GO 20-637 Date: February 2018).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BÖ, EY, NE, HKÜ, DD, UÖ, NK; data collection: BÖ, DAT, BS, CC; analysis and interpretation of results: BÖ, HKÜ, EY, DD, UÖ, NK; draft manuscript preparation: BÖ, EY, HKÜ, NE. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Paranjape SM, Mogayzel PJ. Cystic fibrosis in the era of precision medicine. Paediatr Respir Rev 2018; 25: 64-72. https://doi.org/10.1016/j.prrv.2017.03.001
2. Fowler WS, Cornish ER, Kety SS. Lung function studies. VIII. Analysis of alveolar ventilation by pulmonary N2 clearance curves. J Clin Invest 1952; 31: 40-50. https://doi.org/10.1172/JCI102575
3. Fretzayas A, Douros K, Moustaki M, Loukou I. Applications of lung clearance index in monitoring children with cystic fibrosis. World J Clin Pediatr 2019; 8: 15-22. https://doi.org/10.5409/wjcp.v8.i2.15
4. Harun SN, Wainwright C, Klein K, Hennig S. A systematic review of studies examining the rate of lung function decline in patients with cystic fibrosis. Paediatr Respir Rev 2016; 20: 55-66. https://doi.org/10.1016/j.prrv.2016.03.002
5. Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. J Pediatr 1998; 132: 589-595. https://doi.org/10.1016/s0022-3476(98)70344-0
6. Malhotra RK, Indrayan A. A simple nomogram for sample size for estimating sensitivity and specificity of medical tests. Indian J Ophthalmol 2010; 58: 519-522. https://doi.org/10.4103/0301-4738.71699
7. Ulusal Kistik Fibrozis Kayıt Sistemi: 2019 Yılı Verileri. Available at: https://www.kistikfibrozisturkiye.org/wp-content/uploads/2021/05/KF-Rapor-05.03.2021-1.pdf
8. Fuchs HJ, Borowitz DS, Christiansen DH, et al. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. N Engl J Med 1994; 331: 637-642. https://doi.org/10.1056/NEJM199409083311003
9. Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic Pseudomonas aeruginosa infection in cystic fibrosis patients. J Cyst Fibros 2003; 2: 29-34. https://doi.org/10.1016/S1569-1993(02)00141-8
10. Robinson PD, Latzin P, Verbanck S, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. Eur Respir J 2013; 41: 507-522. https://doi.org/10.1183/09031936.00069712
11. Jensen R, Stanojevic S, Klingel M, et al. A systematic approach to multiple breath nitrogen washout test quality. PLoS One 2016; 11: e0157523. https://doi.org/10.1371/journal.pone.0157523
12. Robinson PD, Latzin P, Ramsey KA, et al. Preschool multiple-breath washout testing. An official American Thoracic Society technical statement. Am J Respir Crit Care Med 2018; 197: e1-e19. https://doi.org/10.1164/rccm.201801-0074ST

13. Rosenfeld M, Allen J, Arets BHGM, et al. An official American Thoracic Society workshop report: optimal lung function tests for monitoring cystic fibrosis, bronchopulmonary dysplasia, and recurrent wheezing in children less than 6 years of age. Ann Am Thorac Soc 2013; 10: S1-S11. https://doi.org/10.1513/AnnalsATS.201301-017ST

14. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005; 26: 319-338. https://doi.org/10.1183/09031936.05.00034805

15. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012; 40: 1324-1343. https://doi.org/10.1183/09031936.00080312

16. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available at: http://www.r-project.org

17. Therneau T, Atkinson B, Ripley B. Rpart: Recursive partitioning and regression trees. R package version 4.1.19. 2022. Available at: https://cran.r-project.org/web/packages/rpart/index.html

18. Torgo L. performanceEstimation: An infrastructure for performance estimation of predictive models. R package version 1.1.0. 2016. Available at: https://cran.r-project.org/web/packages/performanceEstimation/index.html

19. Konstan MW, Morgan WJ, Butler SM, et al. Risk factors for rate of decline in forced expiratory volume in one second in children and adolescents with cystic fibrosis. J Pediatr 2007; 151: 134-139, 139.e1. https://doi.org/10.1016/j.jpeds.2007.03.006

20. Cogen J, Emerson J, Sanders DB, et al. Risk factors for lung function decline in a large cohort of young cystic fibrosis patients. Pediatr Pulmonol 2015; 50: 763-770. https://doi.org/10.1002/ppul.23217

21. Vandenbranden SL, McMullen A, Schechter MS, et al. Lung function decline from adolescence to young adulthood in cystic fibrosis. Pediatr Pulmonol 2012; 47: 135-143. https://doi.org/10.1002/ppul.21526

22. Hollander FM, de Roos NM, Heijerman HGM. The optimal approach to nutrition and cystic fibrosis: latest evidence and recommendations. Curr Opin Pulm Med 2017; 23: 556-561. https://doi.org/10.1097/MCP.0000000000000430

23. Horsley A. Lung clearance index in the assessment of airways disease. Respir Med 2009; 103: 793-799. https://doi.org/10.1016/j.rmed.2009.01.025

24. Fuchs SI, Sturz J, Junge S, Ballmann M, Gappa M. A novel sidestream ultrasonic flow sensor for multiple breath washout in children. Pediatr Pulmonol 2008; 43: 731-738. https://doi.org/10.1002/ppul.20825

25. Anagnostopoulou P, Latzin P, Jensen R, et al. Normative data for multiple breath washout outcomes in school-aged Caucasian children. Eur Respir J 2020; 55: 1901302. https://doi.org/10.1183/13993003.01302-2019

26. Ellemunter H, Fuchs SI, Unsinn KM, et al. Sensitivity of lung clearance index and chest computed tomography in early CF lung disease. Respir Med 2010; 104: 1834-1842. https://doi.org/10.1016/j.rmed.2010.06.010

27. Kraemer R, Blum A, Schibler A, Ammann RA, Gallati S. Ventilation inhomogeneities in relation to standard lung function in patients with cystic fibrosis. Am J Respir Crit Care Med 2005; 171: 371-378. https://doi.org/10.1164/rccm.200407-948OC

28. Aurora P, Stanojevic S, Wade A, et al. Lung clearance index at 4 years predicts subsequent lung function in children with cystic fibrosis. Am J Respir Crit Care Med 2011; 183: 752-758. https://doi.org/10.1164/rccm.200911-1646OC

29. Sonneveld N, Stanojevic S, Amin R, et al. Lung clearance index in cystic fibrosis subjects treated for pulmonary exacerbations. Eur Respir J 2015; 46: 1055-1064. https://doi.org/10.1183/09031936.00211914

30. Vermeulen F, Proesmans M, Boon M, Havermans T, De Boeck K. Lung clearance index predicts pulmonary exacerbations in young patients with cystic fibrosis. Thorax 2014; 69: 39-45. https://doi.org/10.1136/thoraxjnl-2013-203807

31. Amin R, Subbarao P, Jabar A, et al. Hypertonic saline improves the LCI in paediatric patients with CF with normal lung function. Thorax 2010; 65: 379-383. https://doi.org/10.1136/thx.2009.125831

32. Lombardi E, Gambazza S, Pradal U, Braggion C. Lung clearance index in subjects with cystic fibrosis in Italy. Ital J Pediatr 2019; 45: 56. https://doi.org/10.1186/s13052-019-0647-5

33. Kurz JM, Ramsey KA, Rodriguez R, et al. Association of lung clearance index with survival in individuals with cystic fibrosis. Eur Respir J 2022; 59: 2100432. https://doi.org/10.1183/13993003.00432-2021

34. Perrem L, Rayment JH, Ratjen F. The lung clearance index as a monitoring tool in cystic fibrosis: ready for the clinic? Curr Opin Pulm Med 2018; 24: 579-585. https://doi.org/10.1097/MCP.0000000000000515

35. Oude Engberink E, Ratjen F, Davis SD, Retsch-Bogart G, Amin R, Stanojevic S. Inter-test reproducibility of the lung clearance index measured by multiple breath washout. Eur Respir J 2017; 50: 1700433. https://doi.org/10.1183/13993003.00433-2017