Supplementary Material

Quantification of Lung Inflammation in Cystic Fibrosis during Respiratory Tract Exacerbation using Diffusion-Weighted Magnetic Resonance Imaging

Methods

This was a cross-sectional observational study of patients with cystic fibrosis (CF) with and without respiratory tract exacerbation (RTE). All subjects with RTE attended on a two occasions, whilst admitted for RTE (baseline) and after intravenous antibiotic therapy (follow-up), and underwent a series of clinical, lung function and radiological examinations during the same visit. Similarly, control subjects repeated the study protocol over the same median time gap as the RTE group. Visits took place between September 2011 – September 2013.

This study was approved by the Ca'Foncello Regional Hospital Ethics Committee, protocol n. 314/AULSS9. Parents/guardians signed informed consent and paediatric subjects provided assent.

Patient selection

Respiratory tract exacerbation group

No universally accepted definition of a respiratory tract exacerbation (RTE) exists. A general definition, described as "clinical need for additional treatment as indicated by acute changes in clinical parameters", has been recently adopted by the Eurocare CF Working Group.[1] These acute changes during RTE include: change in sputum; increased cough; increased malaise; fatigue or lethargy; anorexia or weight loss; decrease in spirometry outcomes by 10% or more or radiographic changes; and increased dyspnea.

These changes are also used by the Rosenfeld criteria to score the clinically likelihood of RTE in patient with CF (Tab. S1)[2]. All subjects enrolled in the study were scored with the Rosenfeld criteria for RTE both at baseline at follow-up in.

Table S1 Rosenfeld criteria for respiratory tract exacerbations

Rosenfeld Criteria	PEX scoring system
• Reduced exercise tolerance • Increased cough • Increased sputum/cough congestion • School or work absenteeism • Increased adventitial sounds on lung examination • Reduced appetite • Reduced FEV_1 (≥10% predicted)	• Each criterion is assigned a coefficient • PEX score is the sum of the coefficient per each criterion when present • Threshold value to define RTE is 2.6

PEX=pulmonary exacerbation scoring system, RTE=respiratory tract exacerbation, FEV_1= Forced expiratory volume in 1 sec

Clinically stable CF patients – Control group

Clinically stable patients with CF were asked to participate to the study at the annual follow-up visit. Clinically stability was defined when patient had no signs or symptoms of RTE in the previous 6 month to the visit. Each control subject was selected after inclusion of an RTE group subject and matched for age, sex, and mean of FEV_1 in the previous 6 months before the visit.

Study protocol

The study protocol consisted of clinical examination, spirometry and magnetic resonance imaging (MRI). Each RTE subject performed the study protocol at admission (baseline) and after intravenous antibiotic treatment (follow-up). Each control subject performed the study protocol twice with a similar median time gap (~ 20 days) of RTE subjects.

Clinical examination

Clinical examination started with collection of recent patient history, in particular variation on coughing, amount and color of sputum, dyspnea, exercise tolerance, and work or school absenteeism. Physical exam consisted of heart and respiratory rate measurements, O_2 saturation, weight and height recording for body mass index measurement, oral and ear inspection, cardiac and lung auscultation. During the clinical examination, was also performed the PEX score to define whether the subject with CF had or not RTE.

Spirometry

Repeated spirometry efforts were performed until 3 acceptable maximal maneuvers were obtained according to "ATS/ERS guidelines" using a Masterscope (Jaeger-Carefusion, Hochberg, Germany) spirometer[3]. When spirometry traces showed evidence of incomplete expiration, cough, and air leak were repeated. Acceptable repeatability was obtained when the difference between the largest and next largest maneuvers was ≤ 100 ml or 5% depending on which was the larger value. The recorded result for Forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) was the highest achieved across all technically correct efforts, with the ratio calculated from this FEV_1/FVC and reduction in forced expiratory flow at 25-75% of the FVC (FEF_{25-75}) were expressed as % of predicted value.

MRI

MRI was performed at 1.5 Tesla (MAGNETOM Avanto, Siemens Healthcare, Erlangen, Germany) using morphological and functional sequences.

Morphological sequences included axial and coronal end-expiratory triggered Periodically Rotated Overlapping ParallEL Lines with Enhanced Reconstruction (PROPELLER; SIEMENS brand name, BLADE) with proton density (PD) weighting (TR/TE/alpha/TA: 710/26 ms/150°/4' 25'', voxel size 1 x 1 x 6 mm^3; bandwidth 601 Hz/pixel, slice gap 0 mm, average n=4)[4], and breath-hold spirometry gated axial and coronal steady state free precession (SSFP; SIEMENS brand name, TrueFISP) with T_2/T_1 weighting (TR/TE/alpha/TA: 229/0.99 ms/66°/14'', voxel size 2.3 x

2.3 x 6 mm^3; bandwidth 1291 Hz/pixel, slice gap 0 mm, average n=1). For the breath-hold MRI acquisitions, subjects were trained to obtain a maximal breath hold time of 15 seconds at ≥95% Total Lung capacity (TLC) and at ≥ 90% residual volume (RV) using an MRI compatible spirometer (Software B3BOX, Biomedin, Padova, Italy).

The functional data were acquired in the axial plane using a diffusion-weighted (DW) single shot echo-planar imaging (EPI) sequence prototype (TR/TE = 4800ms/54ms, voxel size 2.5 x 2.5 x 6 mm^3; bandwidth 1644 Hz/pixel, slice gap 1.8 mm, average n=3) with end-expiratory triggering. Eleven b-values at different time intervals (0, 10, 20, 30, 50, 70, 100, 150, 200, 400, 800 s/mm^2) were acquired in three orthogonal directions. The entire MRI scan required approximately 25-30 min.

Image analysis

Image analysis was conducted using the picture archiving and communication system (PACS) SYNAPSE (Fujifilm Healthcare Europe, Dusseldorf, Germany). MRI images were anonymized and evaluated in random order by two radiologists (V.T. and S.B.), with 4 and 8 years of experience in thoracic MRI scoring respectively. Both radiologists were blinded to any clinical information and to each other's MRI assessment.

Morphological scoring system: CF-MRI scoring system

Morphological images BLADE and TrueFISP were scored with the Cystic Fibrosis MRI scoring system (CF-MRI), a scoring system derived from the Brody scoring system and adapted to MRI[4,5]. This scoring system evaluates the five lung lobes and lingula as the sixth lobe. The CF-MRI scores assess the following components: bronchiectasis, mucus plug, parenchymal changes (atelectasis, consolidation, ground-glass), trapped air. Differently from CT, air wall thickening is combined with bronchiectasis in the CF-MRI, because of the lower spatial resolution of MRI[4]. For the same reason, the pattern of trapped air is not scored in CF-MRI, as this has previously been shown to be unreliable[4]. A composite score for each component is calculated and expressed as a percentage of a maximum score, on a 0–100 scale. The higher the score the more severe the disease.

Diffusion Weighted Magnetic Resonance Imaging (DW-MRI) Analysis

DW images and Apparent Diffusion Coefficient (ADC) maps: qualitative and quantitative analysis

DW-MRI measures the random Brownian motion of the extra-cellular water molecules in the body. In biologic tissues, this motion is dependent and limited by interactions with cell membranes and macromolecules. In dense cellular tissues or in case of cellular swelling this water motion is reduced or "restricted", because of reduced extracellular space and by cell membranes. Tumors, edema and inflammation are all characterized by restricted diffusion.

DW-MRI provides two types of images: the native DW images, which are repeated at least twice, varying a parameter in the sequence called "b value", and the post-processed Apparent Diffusion Coefficient (ADC) map.

The sensitivity of the DW-MRI technique to water motion depends on scanner's parameters, which are summarized by the b-value, expressed as sec/mm^2 [6,7]. Water molecules with a large degree of motion (i.e. fluid) will have a fast DWI signal decay with high b-values (>700 s/mm^2). Conversely, slow-moving water molecules show a lower DWI signal decay with large b-values. DW-MRI is typically performed using at least two b values to enable meaningful interpretation[8]. The interpretation of the relative attenuation of DWI signal intensity on images obtained at different b values, allows the analysis of different tissue components. For instance, in a tumor with cystic and solid components, the cystic portion will show greater signal decay on high b-value images because water diffusion is less restricted. By contrast, the more cellular solid portion will show relatively high signal intensity at high b-value[8]. Therefore, in case of tumors and inflammation high DWI signal (bright) can be visually assessed at high b-values. However, this qualitative visual assessment is limited by the T2 shine-through artefact, where tissues with long T2-relaxation time have high DWI signal at high b-value, despite normal water diffusion[7]. This potential source of error can be eliminated by using an apparent diffusion coefficient ADC map, which is an average image obtained by DWI signal at different b-values. Areas of restricted diffusion in highly cellular tissue have low ADC values compared with less cellular areas that return higher ADC values. It is important to notice that areas of restricted diffusion will appear as low-signal intensity areas (dark) on ADC map, and as high-signal intensity areas (bright,) on DW images (opposite to ADC maps). Hence, ADC maps are complementary to DW images for a correct interpretation of DWI signal.

In this study, we combined a semi-quantitative scoring system to score DW-MR images and quantitative measurement performed on ADC maps. The DWI scoring system presented in table S2 describes how the lesions with high-signal intensity on DW-MR images with high b value, defined as hotspots, were quantified. This method proved to be reliable in previous studies, with high sensitivity and specificity[9–12]. A minimum of two b-values should be used to assess mono-compartmental diffusion analysis, such as 0-800 s/mm^2 [8]. More sophisticated analysis (bi-compartmental) can be obtained by using Intravoxel incoherent motion analysis (IVIM), which requires a higher b-values sampling between 0-200 s/mm^2 (i.e. 0-25-50-100-150-200 s/mm^2) and larger steps after 200 s/mm^2 (i.e. 200-400-600-800-1000)[13,14]. In this study, we did not perform IVIM analysis, because DW-MRI data needed image registration post-processing for reliably performing IVIM technique. At the time of the study, we did not have dedicated software for imaging registration.

Table S2 – DW-MRI analysis

Parameter	Score
Presence of hotspot	• Score 1: presence of hotspots greater than 5% of lobe volume • Score 0: no hotspot
Extension	• Score 1: hotspots occupying a volume smaller than 33% of the lobe • Score 2= hotspots occupying a volume between 33% and 67% of the lobe • Score 3: hotspots occupying a volume greater than 67% of the lobe
Signal Intensity (SI) dominant hotspot	• Score 1: SI hotspots greater than SI cord spine • Score 0: SI hotspots smaller than SI cord spine
Mean signal intensity (SI_{mean})	• Score 1: mean SI hotspots within a lobe is greater than SI of cord spine • Score 0: mean SI hotspots within a lobe is smaller than SI of cord spine
Sub-score A	• A = (E + SI)
Sub-score B	• B = (E + SI_{mean})
Total DWI score (DWI)	• DWI = P+A+B

DW images were scored using a semi-quantitative scoring system which assesses each lobe using the following parameters: presence of hotspot (P), extension of hotspot (E), signal intensity of the dominant hotspot (SI) and average signal intensity of the hotspots within a lobe (SI_{mean}). For each lobe, we obtained two sub-scores: A = (E + SI) and B = (E + SI_{mean}). The total DWI score is the sum of P + A + B, and the maximum possible score is 54. The DW images with the highest b-value (b= 800 s/mm^2) were scored.

ADC map were used to objectively quantify diffusion in those dominant pulmonary lesions, which showed restricted diffusion at high b-values[15]. The ADC maps were generated automatically from each DW image by the MR system software using all b-values (Syngo software version VB19, SIEMENS, Erlangen, Germany).

A region-of-interest (ROI) was positioned in these dominant lesions with restricted diffusion on ADC maps, using a freehand tracing tool, at baseline and follow-up MR examination. A dominant lesion was defined according two criteria: size, (the largest lesion within a lobe,) and signal intensity (SI) (the lesion with DWI SI higher than the spine at b=800 s/mm^2). Adjacent vessels and areas with susceptibility artifact caused by air-tissue interface were excluded from image analysis. ROI covering the entire size of the hotspot was placed in the same location on ADC

maps from MRI at baseline and follow-up in both groups. When the hotspot was not visible in the follow-up examination, the same ROI size and location of the baseline examination was kept to compute ADC. When no hotspots were identified (i.e. control group), three ROIs of 100 mm^2 were randomly positioned in the lung parenchyma and the mean value was used as ADC value for that subject.

Results

Score reliability

Table S3 Inter and intra-observer agreement for DWI score at baseline and follow-up MRI

	Inter-observer agreement		Intra-observer agreement	
Score	**ICC baseline MRI**	**ICC follow-up MRI**	**ICC baseline MRI**	**ICC follow-up MRI**
Sub-score A	0.918	0.904	0.947	0.920
Sub-score B	0.895	0.873	0.936	0.894
Total DWI score	0.904	0.877	0.939	0.9

ICC=intra-class correlation coefficient, MRI=magnetic resonance imaging. For definition of sub-score A, sub-score B and total DWI score, see Table S2.

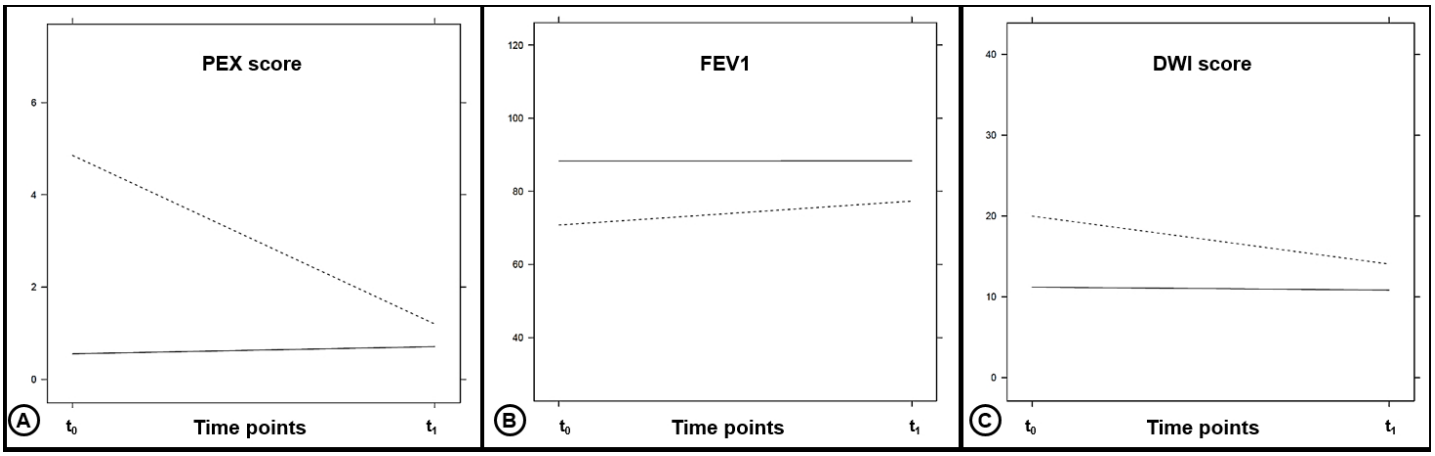

Figure S1. Changes in A) pulmonary exacerbation score (PEX score), B) forced expiratory volume in 1 second (FEV_1) and C) total diffusion-weighted imaging score (DWI score) between case (dotted lines) and control (continuous lines) groups. X-axis represents baseline (t_0) and follow-up (t_1) time points, while the y-axis of each plot represents unit of measure for each variable. Note that RTE group (dotted lines) has significant changes in PEX score, FEV_1 and DWI score, while the control group (continuous line) remains stable.

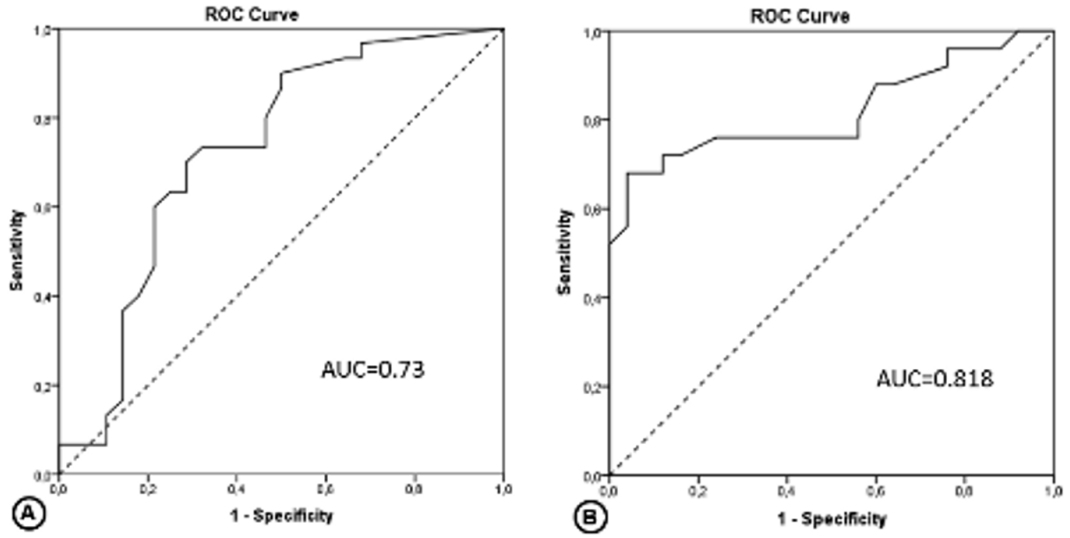

Figure S2. Receiver operating characteristic (ROC) curves for DWI score (A) and ADC (B). AUC values between 0.90-1, 0.80-0.90, 0.70-0.80, 0.60-0.70 correspond to an excellent, good, fair and poor accuracy respectively[16]. Note higher diagnostic accuracy of ADC measurements than DWI score to differentiate patient with CF and respiratory tract exacerbation.

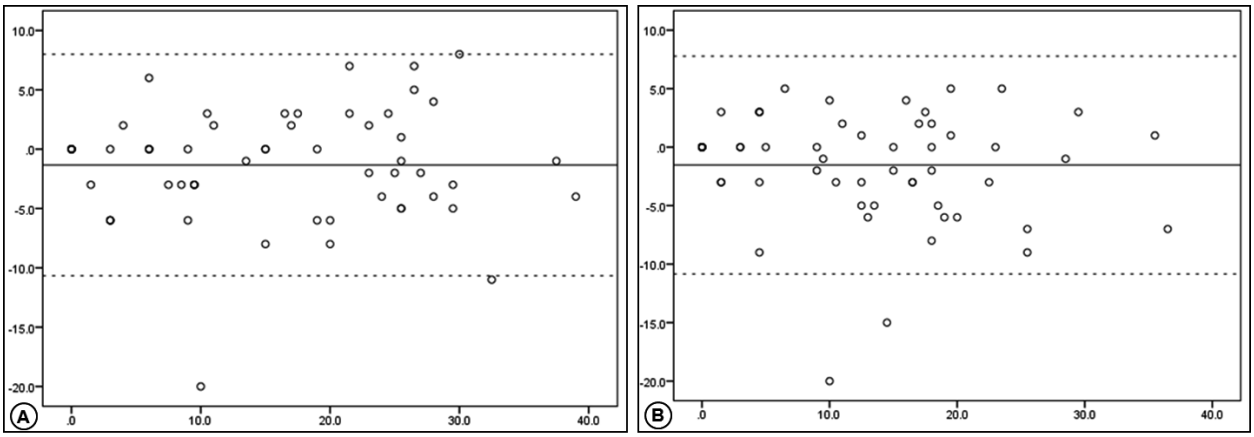

Figure S3. Inter-observer agreement for total DWI scores. Bland-Altman plot of inter-observer agreement (observer 1 versus observer 2) at A) baseline and B) follow-up MRI. Note that mean differences are close to zero and that all observations are around the mean.

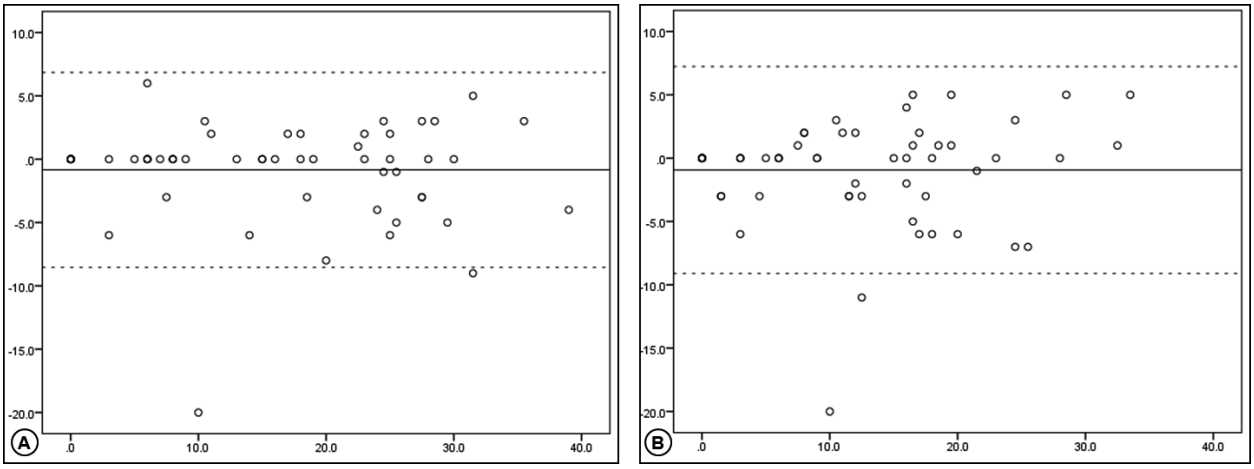

Figure S4. Intra-observer agreement for total DWI scores. Bland-Altman plot of intra-observer agreement (observer 1, one month time gap between scores) at A) baseline and B) follow-up MRI. Note that mean differences are close to zero and that all observations are around the mean.

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