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Intestinal current measurement (ICM) as a new diagnostic test for cystic fibrosis (CF)

Malena Cohen-Cymberknoh[1,2], Yasmin Yaakov[1,3], Eitan Kerem[1,2], David Shoseyov[1,2], Joseph Rivlin[4], Lea Bentur[5], Elie Picard[6], Micha Aviram[7], Michael Wilschanski[1,3]. *[1]CF Center, [2]Department of Pediatrics, [3]Department of Pediatric Gastroenterology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [4]CF Center, Carmel Medical Center, Haifa, Israel; [5]CF Center, Rambam Medical Center, Haifa, Israel; [6]CF Center, Shaare Zedek Medical Center, Jerusalem, Israel; [7]CF Center, Soroka Medical Center, Beer Sheva, Israel*

Background: Like the nasal potential difference (NPD) test, ICM may be useful for the diagnosis of atypical CF. However, ICM is easily applicable at all ages.
Aim: To assess the diagnostic reliability of ICM in a large cohort of CF, healthy control and patients with questionable CF.
Methods: Rectal biopsies were taken from 3 groups: known CF patients, healthy controls and patients with questionable CF. The last group had a variety of symptoms suggestive of CF: recurrent pneumonia, unexplained bronchiectasis, chronic diarrhea and/or failure to thrive. ICMs were performed using standard protocols by mounting the rectal biopsy in an Ussing chamber and sequentially adding secretagogues while recording current changes.
Results: 17 known CF patients and 16 control patients were examined and have remarkably different results (all results are presented as $\mu A/cm^2$): carbachol 16±7, histamine 13±9 and forskolin 4.8±4 for healthy control group and carbachol -3.7±6.8 ($p<0.0001$) histamine -3.1±2.7 ($p<0.0001$) and forskolin 0.2±0.4 ($p=0.0004$) for the CF group. The suggested reference values are: +3.75, +0.25, +1.32 for carbachol, histamine and forskolin, respectively. The combination score (the sum of the 3 secretagogues) differentiates normal from abnormal ICM (ROC Curve analysis, area under the curve =1.00, both sensitivity and specificity are 100%). This statistical model was applied to 70 patients suspected for CF and revealed that 59 patients had normal and 11 patients had abnormal ICM results.
Conclusion: In this study we have shown that ICM tests may be useful to differentiate between CF and non-CF patients and may be included in diagnostic algorithms. Larger studies are needed to confirm these results.

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Regulation of ion transporters and airway surface dynamics by lipoxin in cystic fibrosis bronchial epithelium

Mazen Al-Alawi[1], Valia Verriere[1], Richard Chostello[2], Valerie Urbach[1], Brian Harvey[1]. *[1]Department of Molecular Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland; [2]Department of Respiratory Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland*

Aims: We have investigated the role of the endogenous anti-inflammatory lipoxin LXA_4 in modulating Cl^- secretion and Na^+ absorption, airway surface liquid height (ASLh) and ciliary beat frequency (CBF) in CF and non-CF bronchial epithelia.
Methods: CF (CuFi-1) and non-CF (NuLi-1) bronchial epithelial cell lines were grown under an air-surface liquid interface into well-differentiated epithelia. ASLh and CBF were measured using confocal fluorescence microscopy and ion transport using patch-clamp and short-circuit current techniques.
Results: LXA_4 (1nM) treatment for 15 minutes, increased ASLh by 47.5±0.5% and 103.0±3.0% in NuLi and CuFi epithelia respectively ($P<0.001$, n=18). The stimulatory effect of LXA_4 on ASLh was sustained over 24 hours in the CF epithelia and was inhibited by the following pre-treatments: bumetanide, amiloride, Boc-2 (LXA_4 receptor antagonist), reactive blue (P2Y receptor antagonist) and extracellular hexokinase (ATP hydrolysis). LXA_4 stimulated CBF, intracellular Ca^{2+} mobilization, Cl^- secretion and inhibited Na^+ absorption in the CF epithelia.
Conclusions: These effects of lipoxin involving the FPR2 receptor, apical ATP release, purinoreceptor activation, inhibition of Na^+ absorption and stimulation of Cl^- secretion to enhance airway surface liquid dynamics open up a new therapeutic avenue to promote mucociliary clearance in cystic fibrosis airways.

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Deficient production of IFN-stimulated genes upon rhinovirus infection in cystic fibrosis airway epithelial cells

Elisabeth Kieninger[1], Marjolaine Vareille[1], Marco P. Alves[1], Brigitte S. Kopf[1], Thomas Geiser[2], Sebastian L. Johnston[3], Michael R. Edwards[3], Nicolas Regamey[1]. *[1]Division of Paediatric Respiratory Medicine, University Children's Hospital, Bern, Switzerland; [2]Division of Pulmonary Diseases, University Hospital of Bern, Bern, Switzerland; [3]Department of Respiratory Medicine, National Heart & Lung Institute, Imperial College London, London, United Kingdom*

Background: Rhinoviruses (RVs) are important triggers of pulmonary exacerbations and possible contributors to long-term respiratory morbidity in cystic fibrosis (CF), but mechanisms leading to RV-induced CF exacerbations are poorly understood. We recently described deficient innate immune responses to RV infection in CF characterized by impaired type I and III interferon (IFN) production and increased virus replication.
To study downstream effects of impaired IFN induction we investigated the expression of IFN-stimulated genes (ISGs) which are important for the production of antiviral proteins.
Methods: Epithelial CF and non-CF cell lines (UNCCF2T/UNCN2T, CFBE41o-/16HBE14o-) were cultured and infected with RV-16 and -1B at a MOI of 2. Induction of ISGs including MxA, 2',5'-OAS, viperin and NOS2 was assessed by RT-PCR. Exogenous IFN-β and -λ were added before and after infection.
Results: Expression of all ISGs was induced in CF and control cells upon virus infection. CF cells expressed 100-1000 times less ISGs than control cells (all $p<0.05$). ISG expression and RV replication were inversely related (MxA: r=-0.79, p=0.001). There was a positive correlation between ISG expression and IFN-β (2',5'-OAS: r=0.74, p=0.004) and IFN-λ production (NOS2: r=0.65, p=0.01). Exogenous IFN increased levels of ISGs to the level of control cells, with a more pronounced effect of IFN-β.
Conclusions: ISG induction upon RV infection is deficient in CF indicating a profound impairment of the early innate antiviral response. Addition of exogenous IFN restores antiviral pathways in CF, suggesting a potential use of IFNs in the prevention or treatment of RV-induced CF exacerbations.

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Association of FCN1 and FCN2 gene polymorphisms with earlier onset of chronic *pseudomonas aeruginosa (Pa)* colonisation in cystic fibrosis (CF) patients

Filomeen Haerynck[1], Kristel Van Steen[2], Tom Cattaert[2], Bart Loeys[3], Sabine Van Daele[1], Petra Schelstraete[1], Frans De Baets[1]. *[1]Pediatric Pulmonology and Immunology, Ghent University Hospital, Ghent, Belgium; [2]Statistical Genetics, University of Liege, Liege, Belgium; [3]Medical Genetics, Antwerp University Hospital, Antwerp, Belgium*

Background: CF is a multisystem disease with high degree of phenotypic variability especially in lung disease.Modifying genes of innate immunity may be involved in early onset of *Pa* colonisation.
Methods: 82 Single Nucleotide Polymorphisms (SNPs) in 22 genes contributing to the innate immunity (MBL2, MASP (MBL associated serine Protease) 1/2/3, FCN (Ficolin) 1/2, LBP (Lipopolysaccharide-binding Protein), CD14,TLR (Toll-likereceptors 1→10)) were genotyped in a cohort of 116 CF patients. (age 6-44 years) Association survival analysis (Kaplan Meier and Cox regression) using additive, recessive, dominant and codominant model was performed looking for an association between SNPs and age of onset of *Pa* colonisation in all CF patients.
Results: CF patients being heterozygous or homozygous for the mutant allel of both linked SNPs FCN1 (promoter) (A/G) and FCN1 (Q272Q) (exon 9) (G>A) are earlier colonised with *Pa* (p=0,016,p=0,026 resp). Earlier onset of *Pa* colonisation is seen in CF patients homozygous for mutant allel of -64A>C polymorphism FCN2 (promoter) (p=0,0031) and in patients having at least one mutant allel of the linked S258A (G>T)) polymorphism FCN2 (p=0,0057).
CF patients heterozygous for mutant allel of TLR10 (rs7694115) (promoter) (T>C) (p=0,026) and linked SNP (rs11096957) (ex3) (N241H) (p=0,0113) and SNPs (rs11466645) (pro) (T>A) (p=0,0068), (rs11096956) (ex3) (P344P) (p=0,0067) are significantly later colonised with *Pa*.
Conclusion: Mutant allel of SNPs FCN1 (pro and Q272Q) and FCN2 (-64A>C and S258A) is significantly associated with earlier *Pa* colonisation. Mutant allel of polymorphism of TLR10 is associated with later onset of *Pa* colonisation.

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The polyamine spermine is increased in cystic fibrosis airway secretions

Hartmut Grasemann[1], Darakhshanda Shehnaz[1], Paul Pencharz[2], Felix Ratjen[1]. *[1]Pediatrics, Respiratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada; [2]Gastroenterology, Hepatology and Nutrition, The Hospital for Sick Children, Toronto, ON, Canada*

Rationale: Sputum arginase contributes to the nitric oxide (NO) deficiency in cystic fibrosis (CF) airways. Ornithine, the product of arginase activity, is the precursor of polyamines, which may play a role in the pulmonary response to injury and remodeling.
Objective: To measure concentrations of spermine in sputum of CF patients.
Methods: Using mass spectrometry, spermine was measured in sputum of clinically stable patients with CF (n=10), CF patients before and after antibiotic treatment for a pulmonary exacerbation (N=10) and healthy controls (n=10). CF patients were 7-17 years of age. Mean FEV1 in the stable CF patients was 80.4 (range 47-117)% of predicted values. FEV1 in CF patients presenting with a pulmonary exacerbation was 50.8±3.5 (range 36-69)% of predicted and improved by 13.5 (±2.8)% with treatment.
Results: Mean (±SEM) spermine concentration in sputum was significantly higher in stable CF than controls (1.71±0.59 vs. 0.22±0.05 μmol/ml, p=0.02). Spermine concentrations were highest in CF patients presenting with a pulmonary exacerbation (9.10±1.62 μmol/ml) and decreased to levels similar to stable CF (1.68±0.33 μmol/ml, $p<0.001$, paired t-test), but remained significantly increased when compared to controls ($p<0.001$). The change in spermine concentrations during treatment for a pulmonary exacerbation correlated significantly with the

changes in sputum arginase activity (R=0.65, p<0.05) and ornithine concentrations (R=0.8, p=0.01).
Conclusions: Spermine is significantly increased in the airways of patients with CF and linked to increase arginase activity. Further studies of the role of the polyamines for CF lung disease are warranted, as they may contribute to airways obstruction and remodeling.

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Regulation of corticosteroid binding globulin (CBG) in the inflammatory context of cystic fibrosis
Jessica Taytard[1], Carine Rebeyrol[1], Dominique Debray[1], Annick Clement[1,2], Nicolas Chignard[1], Philippe Le Rouzic[1]. *[1]UMR_S938, Inserm, UPMC Univ Paris 06, Paris, France; [2]Paediatric Pulmonology, Hôptital Armand Trousseau, APHP, Paris, France*

Background: Cystic Fibrosis (CF) is characterised by chronic lung inflammation. In CF, glucocorticoids (GC) are a widely used therapeutic tool. However, their efficiency, and the benefit/risk ratio are still discussed. In plasma, 90% of GC is bound to the chaperone protein CBG which regulates its bio-disponibility. CBG is mainly produced by the liver. Recent works enlightened the fact that, more than a simple carrier protein, CBG could also address GC specifically to the inflammation site, thereby modulating the response to GC in an inflammatory context.
Objectives: Study the expression and regulation of CBG in the liver and assess its pulmonary expression in the inflammatory context of CF.
Methods: *Hepatic levels of CBG:*
– Biopsies from healthy donors, cirrhotic CF and non CF patients: measure of the transcripts levels of CBG and interleukin-6.
– Hepatocarcinoma derived cell-lines Hep3B and HepG2: regulation of CBG expression.
Lung levels of CBG:
– Lung biopsies; expression of CBG
– Bronchial epithelial cell lines; regulation of CBG expression
Results: We show an increase in CBG expression:
– in the liver and lung of CF patients.
– in the hepatic and lung cell lines in an inflammatory context.
GC has no effects on CBG expression in hepatic cell lines, but increases CBG levels in the lung cell lines.
Discussion: We show stimulation of the expression of CBG in the inflammatory context of CF. Comparative results from hepatic and lung cell lines enlight a different regulation of CBG expression. Overall, increase in CBG expression in CF could mean initially a decrease in GC bio-disponibility but, ultimately, an enhanced corticosteroid half life and possible prolonged effects.

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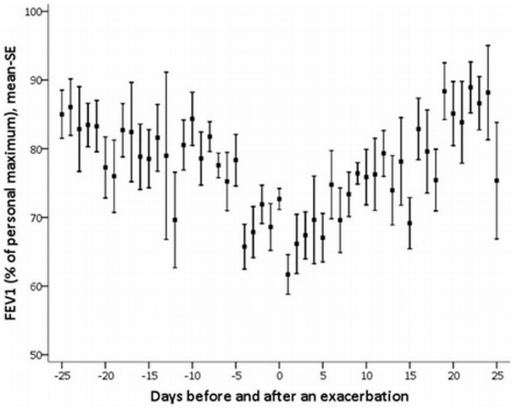
The value of lung function monitoring by means of a home monitor in patients with cystic fibrosis
Charlotte Robroeks, Quirijn Jöbsis, Han Hendriks, Edward Dompeling. *Paediatric Pulmonology, Maastricht University Medical Centre, Maastricht, Netherlands*

Background: Morbidity and mortality in cystic fibrosis (CF) are mainly caused by pulmonary complications. Early recognition of an exacerbation enables early intervention in CF management. We hypothesized that lung function indices drop significantly before an exacerbation is clinically evident, and, that CF exacerbations can be predicted when lung function is assessed by means of a home monitor.
Aims: To study the course of lung function indices before a CF exacerbation, and, to study the potential value of home monitors to predict pulmonary CF exacerbations.
Methods: During this one year longitudinal study, 26 CF patients (aged 6-48yrs) used a home monitor (AM1, Carefusion®, Germany) in order to perform dynamic spirometry three times weekly. In addition, patients visited the outpatient clinic every two months and during an exacerbation 4 extra visits were planned.
Results: 17 Of the 26 included patients experienced an exacerbation. 10 Days to 4 days before the exacerbation, FEV_1 of the personal maximum during the study (mean±standard error) drops from 84±4% to 65±3% (Friedman, p<0.001).
Conclusions: This study showed that lung function already deteriorates 10 to 4 days before a pulmonary CF exacerbation is clinically evident. Therefore, assessments of spirometry by means of a home monitor may predict CF exacerbations. This may be of help for the early treatment and prevention of exacerbations in CF patients.



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Indicators of pulmonary exacerbation in adults with cystic fibrosis (CF)
Fiona McCourt[1], Brenda O'Neill[1], Ian Logan[1], Janice Abbott[2], Evie McCrum-Gardner[1], Stephanie McKeown[1], Judy Bradley[1], Stuart Elborn[3]. *[1]Health and Rehabilitation Science Research Institute, University of Ulster, Jordanstown, Northern Ireland, United Kingdom; [2]School of Psychology, University of Central Lancashire, Preston, England, United Kingdom; [3]Centre for Infection and Immunity, Queen's University, Belfast, Northern Ireland, United Kingdom*

Background: Exacerbations negatively impact adults with CF. No agreed definition of an exacerbation exists.
Aim: To establish agreement on indicators of an exacerbation in adults with CF.
Methods: 2 parallel Delphi web surveys in 13 UK and Ireland CF centres. Delphi 1: 31 adults with CF (FEV_1 <80%) with at least one exacerbation in the previous 12 months. Delphi 2: 38 CF clinicians involved in diagnosing CF exacerbations. *Round 1:* A list of potential indicators of exacerbations were extracted from the literature, consultation with clinicians and adults with CF and 48 statements developed. *Round 2:* Participants rated their level of agreement with each statement. Round 3: Currently active. Statements not reaching consensus in Round 2 were presented to participants to re-rate. Consensus of 75% agreement was applied to all statements.
Results: Round 2, Adults with CF: 21 statements reached consensus. The top 3 were: "More shortness of breath than usual", "Feeling the need to do more airway clearance than usual", "A large decrease in lung function". Round 2, CF clinicians: 23 statements reached consensus. The top 3 were: "Increased sputum", "A large decrease in lung function (>10% FEV_1)", "Increased coughing". Of statements reaching consensus 16 were common in both groups.
Conclusions: This study ascertained important indicators of exacerbation from patients and clinicians. Reflection on the results of both Delphi studies will allow comparisons to be drawn on the perspective of CF adults versus CF clinicians to identify the areas where there are differences and also the areas where there are strong agreement.