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

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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.

Aims: To assess the diagnostic reliability of ICM in a large cohort of CF, healthy control and patients with questionable CF.

Methods: Retinal 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: Of 16 patients (10 control patients were examined and have remarkably different results (all results are presented as μA/cm²): carbachol 16±6, histamine 13±9 and forskolin 4.8±4 for healthy control group and carbachol 3.7±3.6, histamine -3.1±2.7, forskolin 0.2±0.4 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 : 0.89, 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.

Conclusions: 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.

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

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Background: Recent studies indicated that the lipoxin A4/LXA4 receptor (LXA4R) family members may play a role in the pulmonary and airway defense.

Aims: To study the role of lipoxin A4 in regulating ion transporters and the airway surface liquid dynamics in CF patients.

Methods: Airway tissue was obtained from CF (CuFi-1) and non-CF (NuLi-1) bronchial epithelial cell lines that were grown under an air-surface liquid interface into well-differentiated epithelia. ASLh was measured using the rhodamine 6G method. Ca2+ mobilization, Cl- secretion and Na+ absorption were measured by the electric current method. ATP-release, purinoreceptor activation and IP3 levels were measured using calcium imaging, fluoride method and Western blot analysis respectively.

Results: CuFi-1 cells expressed significantly higher levels of LXA4R, compared to NuLi-1 cells. CuFi-1 cells expressed significantly higher basal intracellular Ca2+ levels and Cl- secretion leading to enhanced airway surface liquid dynamics open up a new therapeutic avenue to promote mucociliary clearance in cystic fibrosis airways.

Conclusions: These effects of lipoxin involving the LXA4 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.

(7), 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.

Methods: Epithelial CF and non-CF cell lines (UNCCF21T/UNCN2T, CFBE41o- /16HBE14o-) were cultured and infected with RV-1 and -1B at a MOI of 2. Infection of ISGs including Mea, NOS2 and FOS were assessed by RT-PCR. Exogenous IFN-β and -α 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 ISG than control cells (all p < 0.05). ISG expression and RV replication were inversely related (r= -0.79, p<0.001). There was a positive correlation between ISG expression and IFN-β and -α production (NOS2: r=0.65, p<0.01). Exogenous IFN increased isoflavin 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.

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

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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 disease.

Methods: 82 Single Nucleotide Polymorphisms (SNPs) in 22 genes contributing to the innate immunity (MBL2, MAS3, MBL associated serine protease) 1/2, FCN (Ficolin) 1/2, LBP (Lipopolysaccharide-binding Protein), CD14, TLR (Toll-like receptors 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 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 allele of both linked SNPs FCN1 (promoter) (A/G) and FCN2 (Q72Q) (exon 9) (G/A) are earlier colonised with Pa (p = 0.001) (Table 1). The onset of Pa colonisation is seen in CF patients homozygous for mutant allele of -64A>C polymorphism FCN2 (promoter) (p =0.003) and in patients having at least one mutant allele of the linked S258A (G>T) polymorphism FCN2 (p =0.005).

CF patients heterozygous for mutant allele of TLR10 (rs67964155) (promoter) (T>C) (p =0.026) and linked SNP (rs11096957) (ex3) (N241H) (p=0.0113) and SNPs rs11466465 (p=0.0086), (rs11096505) (ex3) (P344R) (p=0.0067) are significantly later colonised with Pa.

Conclusion: Mutant allele of SNPs FCN1 (pro and Q72Q) and FCN2 (-64A>C and S258A) is significantly associated with earlier Pa colonisation. Mutant allele of polymorphism of TLR10 is associated with later onset of Pa colonisation.

365 The polyamine spermine is increased in cystic fibrosis airway secretions

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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% (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 (1.7±0.59 vs. 0.22±0.05 μmol/ml, p<0.001). Spermine concentrations were highest in CF patients presenting with a pulmonary exacerbation (9.10±2.8)% of healthy controls. 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
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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.

Conclusions: GC has no effects on CBG expression in hepatic cell lines, but increases CBG – in the hepatic and lung cell lines in an inflammatory context.

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The value of lung function monitoring by means of a home monitor in patients with cystic fibrosis
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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, Carefusin®), 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, FEV1 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)
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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 (FEV1 <80%) with at least one exacerbation in the previous 12 months. Delphi 2: 38 CF clinicians involved in diagnosing CF exacerbations. Delphi 1: 38 statements derived from the literature, consultation with clinicians and adults with CF and 48 statements developed. Delphi 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% FEV1)”, “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.