PEPSIN IN BRONCHOALVEOLAR LAVAGE FLUID IS ASSOCIATED WITH AIRWAY INFLAMMATION IN YOUNG CHILDREN WITH CYSTIC FIBROSIS

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Background: Pulmonary infection and inflammation, characterised by increased neutrophil and interleukin 8 (IL-8) levels in bronchoalveolar lavage fluid (BALF), are the main causes of morbidity and mortality in cystic fibrosis (CF). Although airway infection is a recognised cause of inflammation in CF, inflammation may be present in the absence of infection and therefore other triggers of inflammation need to be considered. The role of aspiration in airway inflammation has not been fully investigated in CF. In animal studies, intratracheal instillation of gastric acid is associated with increased neutrophils and IL-8 in BALF. Pepsin, which is the major gastric protease, has also been recovered from BALF in animals with experimentally induced acid aspiration. **Aims:** To determine whether the presence of pepsin in BALF is associated with airway inflammation in young, clinically well children with CF. **Methods:** Twenty-eight children with CF, (<6 years), underwent flexible bronchoscopy and BAL, when clinically well. Five children had two procedures one year apart. Thirty-three specimens were obtained in total. Single 1ml/kg aliquots of sterile saline, were instilled into the right upper lobe, right middle lobe and left lingular segments and aspirated immediately. BALF specimens were pooled and sent for quantitative microbiological culture, fungal culture, viral immunofluorescence and culture, cell count and differential and IL-8 and pepsin determination. Pepsin was measured by a proteolytic assay involving fluorescein isothiocyanate-labelled casein. **Results:** The median age was 2.3 years and 64% were male. 14 children (50%) had evidence of infection. Pepsin was positive in 9 (32%). Only 2 had a clear history of gastroesophageal reflux. The presence of pepsin was associated with higher neutrophil differential counts, absolute differential counts, total cell count and IL-8 levels. The difference was predominantly seen in non-infected children (see table, median values are shown, Mann-Whitney).

	All Specimens (n = 33)			Non-infected subgroup (n = 16)		
	Pepsin pos (n = 11)	Pepsin neg (n = 22)	P value	Pepsin pos (n = 11)	Pepsin neg (n = 5)	P value
PMN %	60.4	41.5	0.03	55.5	11.5	0.03
Abs PMN$\times 10^3$	336	135	0.03	242	21.7	0.02
TCC$\times 10^3$	510	410	0.07	470	140	0.02
IL-8 pg/ml	4993	2349	0.001	4938	1355	0.01

Conclusion: This preliminary study suggests that pulmonary aspiration, as indicated by the presence of pepsin in BALF, is associated with airway inflammation in young, well CF children. The clinical significance of this finding is being investigated.

CONGENITAL MUSCULAR DYSTROPHY (CMD) DUE TO PRIMARY MEROSIN DEFICIENCY SHOWS A SLOWLY PROGRESSIVE SEVERE RESPIRATORY INSUFFICIENCY

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Introduction: Congenital muscular dystrophies (CMD) are a heterogeneous group of muscular diseases with autosomal recessive inheritance. The most frequent type of CMD is primary merosin deficiency due to mutations in the *LAMA2* gene (type MDC1A). Patients may develop restrictive respiratory insufficiency but there is little data in the literature. **Patients and methods:** We studied the respiratory involvement in 29 patients with MDC1A. Diagnosis was done by genetic analysis or by immunohistochemical plus neuroimaging studies.

Clinical aspects, functional respiratory tests, blood gases and ventilatory techniques were reviewed. Clinical follow-up ranged from 1-45 years (mean 10 years). **Results**: VC decreased progressively during childhood. Mechanical ventilation was required in 62% of the patients. Fifteen patients were on nasal nocturnal ventilation (onset mean age: 10,8 years). Twelve patients were tracheotomized ulteriorly (mean age: 15,5y). Regarding the four patients who were able to walk, three had a milder restrictive insufficiency (VC over 30% at 15 years) and did not required ventilation. The only patient who was ventilated had a dramatic worsening of VC during puberty coinciding with the progression of a severe scoliosis. **Conclusion:** Respiratory insufficiency is a frequent complication of MDC1A and its progression follows a typical slowly progressive pattern. The severity of the progression is related with the motor performance and the orthopaedic complications. Investigation of the respiratory function is of therapeutic importance.

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DIAGNOSIS CYSTIC FIBROSIS?: COMPARISON OF NASAL POTENTIAL DIFFERENCE, INTESTINAL CURRENT MEASUREMENT AND SWEAT CHLORIDE IN PATIENTS WITH BORDERLINE SWEAT TEST AND NON-INFORMATIVE CFTR MUTATION ANALYSIS

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Introduction: Patients with mild or monosymptomatic Cystic Fibrosis (CF) might be difficult to diagnose because of borderline sweat test and non-informative *CFTR* mutation analysis. For intestinal current measurement (ICM) in rectal suction biopsies (Veeze 1994, Bronsveld 2000) as additional diagnostic method to investigate CFTR function, we previously showed sensitivity and specificity of 100% by evaluations of CF (including F508del and rare genotypes with residual chloride secretion) and control. Now we aimed to compare this test with nasal potential difference (nPD) and sweat chloride results in order to establish a most informative diagnostic procedure for patients with borderline diagnostic features. **Methods:** From n = 144 patients (13.1 ± 11.6 yrs.) with mild phenotype who came for diagnostic purposes because of borderline sweat test and inconclusive *CFTR* mutation analysis, n = 34 individuals were investigated by both nPD and ICM. The correlation between the chloride secretory responses (due to CFTR and non-CFTR chloride channels) in both tissues and the correlation to sweat chloride concentration was evaluated. **Results:** In n = 2 patients (sweat chloride 50; 53 mmol/l) the diagnosis CF was confirmed by both nPD and ICM, all other patients showed normal electrophysiological chloride secretion parameters and were therefore excluded from the diagnosis CF. Basal PD in the nPD ($p = 0.001$) as well as the net change in short-circuit current evoked by carbachol ($p = 0.001$) and histamine ($p = 0.02$) in the ICM clearly segregated into CF and non-CF, whereas no correlation with absolute sweat chloride concentrations was seen. **Conclusion:** Chloride conductance phenotypes were consistent in both respiratory and intestinal tissue in this highly informative patient cohort. As nPD can be false negative in special conditions and younger patients might need a sedation, ICM should be applied additionally in cases of borderline phenotype and inconclusive other diagnostic tests.

LEUKOTRIENE RECEPTORS EXPRESSION AND DISTRIBUTION IN TONSILS OF CHILDREN WITH SLEEP APNEA AND RECURRENT INFECTION

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Background: Recurrent tonsillitis and sleep apnea are the major indications for tonsillectomy in children. We hypothesized that the recurrent vibration in the upper airway of children with sleep apnea would promote inflammatory changes in the tonsillar tissue, and lead to up regulation of cys Leukotriene (LT) receptors. **Objective**: To asses expression patterns of the human LT1 and LT2 receptors in children undergoing tonsillectomy, and compare between children with recurrent tonsillar infections (RI) to children with sleep apnea (SA). **Methods**: Tonsillar tissue from children with SA or RI was subjected to quantitative polymerase chain reaction using specific primers for LT1 and LT2 receptors to study gene expression. Immunohistochemistry for topographic distribution pattern, and western blotting for semi quantitative protein expression of LT1 and LT2 receptors were also performed. **Results**: mRNA encoding for expression of LT1 and LT2 receptors was detected in tonsils of all children, and did not differ between RI and SA groups (n = 8/group). Significantly higher expression of LT1 and LT2 receptors emerged in the tonsillar tissue of children with SA, with markedly different topographic distribution pattern for both LT1 and LT2 receptors in SA compared to RI patients. **Conclusion**: LT1 and LT2 receptors are expressed in pediatric tonsillar tissue, are more abundant in SA patients, and reveal disease-dependent topographic patterns of expression. We postulate that leukotriene receptor targeted therapies may have a role in SA.

AIRWAY INFLAMMATION AND REMODELLING IN CHILDREN WITH CYSTIC FIBROSIS

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Introduction: The airway inflammatory response is exaggerated in cystic fibrosis (CF). There is little information on the relationship between inflammation and structural airway changes in early stage disease. We aimed to compare this relationship between CF and other inflammatory lung diseases. **Methods:** Flexible bronchoscopy was performed for clinical reasons in 3 groups of children: i) CF, ii) Primary ciliary dyskinesia (PCD), and iii) recurrent respiratory tract infections (RTI). Broncholaveolar lavage (BAL) was performed in the right middle lobe with endobronchial biopsy in the right lower lobe. BAL underwent quantitative microbiology, total and differential cell count. Reticular basement membrane (RBM) thickness was measured using light microscopy. **Results**: 44 children were studied (23 CF, 6 PCD, 15 RTI. A pathogenic organism was identified on BAL in 18 (8 CF, 4 PCD, 6 RTI). 42 biopsies were available, of which 28 were of sufficient quality for RBM measurement (67%; 14 CF, 5 PCD, 9 RTI). Both CF and PCD groups had pronounced neutrophil (neu) domination in BAL compared to the RTI group ($p < 0.001$). The ratio of neutrophils to bacteria (colony forming units, cfu) in BAL was higher in CF than in RTI, but was similar to that in PCD ($p = 0.08$). RBM was thickened in the CF group but was not predicted by age, bacterial count, or cell counts. Data are expressed as median and [range].

	Age yrs	10^6/ml Cells	Neu %	Neu 10^6/ml	Log_{10} (Neu/cfu)	RBM µm
CF	6.6 [0.3-14.3]	1.7 [0.3-28.5]	55 [0-89]	0.7 [0-23.9]	1.07 [0.21-3.49]	6.4 [3.9-9.1]
PCD	9.8 [5.7-14.8]	2.6 [0.2-14.8]	66 [33-96]	2.3 [0.05-13.8]	0.44 [−0.86-1.02]	5.2 [3.4-9.8]
RTI	3.8 [0.9-13.6]	0.3 [0.2-1.0]	7 [1-69]	0.01 [0-0.2]	−1.04 [−1.70-2.86]	4.5 [3.2-8.4]
p (CF vs RTI)	0.15	<0.001	<0.001	<0.001	<0.05	<0.05

Conclusion: This study provides evidence of remodelling within the airways of children with cystic fibrosis. There are similar cellular changes on BAL in CF and PCD, and RBM thickening therefore may not be directly related to inflammation occurring within the airway lumen.

COMPUTED TOMOGRAPHY AND CORRELATION WITH PULMONARY FUNCTION TEST RESULTS IN PAEDIATRIC SEVERE ACUTE RESPIRATORY SYNDROME (SARS)

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Objective: Severe acute respiratory syndrome (SARS) associated coronavirus causes pulmonary damage. We evaluated thin-section CT scans of SARS affected children and correlated the imaging findings with pulmonary function test results. **Subjects and Methods**: Forty-seven serologically confirmed SARS subjects, at 6 months follow-up, underwent thin-section CT of the thorax during inspiration and expiration. All suitable subjects also underwent pulmonary function tests. A radiologist who was unaware of the pulmonary function test results, evaluated the CT scans for the presence and severity of focal air trapping and residual ground glass appearance. **Results**: CT revealed focal air trapping and residual ground glass appearance in ten and six subjects respectively. The mean values of forced expiratory volume in 1 second (FEV_1), forced mid expiratory flow and forced vital capacity (FVC) were significantly lower for subjects with residual ground glass appearance on CT (mean = 78, 81 and 79% predicted respectively) than for those with focal air trapping (mean = 94, 95 and 98% predicted respectively; $p = 0.001$) or normal CT scan results (mean = 89, 91 and 93% predicted respectively; $p = 0.026$). Combination of air trapping and residual ground glass appearance on CT was seen in three subjects: All three had required oxygen therapy and was subsequently ventilated during the acute phase of SARS infection. **Conclusion**: In SARS affected children and adolescents, focal air trapping and residual ground glass appearance were common findings on thoracic CT scans performed at 6 months. In addition, presence of residual ground glass on CT was associated with significantly lower FEV_1, forced mid expiratory flow and FVC values than those found for subjects in whom CT revealed focal air trapping or no abnormalities. Worse CT scan results were seen in those required oxygen supplementation and ventilation during the acute episode.

LARYNGEAL RESPONSE TO NASAL VENTILATION IN NON-SEDATED LAMBS

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Nasal intermittent positive pressure ventilation (NIPPV) is increasingly used in neonates for respiratory distress syndrome, apneas of prematurity, or as a bridge from endotracheal to spontaneous ventilation. However, while studies in adults have shown that active laryngeal closure can limit alveolar ventilation during NIPPV, no such data are available in neonates. **The aim** of the present study was to assess laryngeal muscle response to NIPPV in lambs. **Methods.** Eight lambs were surgically instrumented at 1–3 days of life for recording sleep stages (electrocorticogram + eye movements), electromyogram (EMG) of a laryngeal adductor (thyro-arytenoid muscle, TA) and abductor muscle (crico-arytenoid muscle, CT), diaphragm (Di) EMG and subglottal pressure (tracheal catheter). Recordings including thoraco-abdominal movements (Respitrace) and mask pressure monitoring were performed 2 days later in non-sedated lambs, using our radiotelemetry equipment. Synchronized, volume-controlled ventilation with 4 cm H_2O PEEP was delivered *via* a nasal mask using a Servo 300, progressively increasing tidal volume from spontaneous ventilation to a maximum of 23.6 ± 3.4 ml/kg. Mean integrated EMG of the TA, CT and Di was measured during inspiration with trans-upper airway pressure for all ventilatory conditions, and analyzed in relation with states of alertness. **Results.** Increasing NIPPV during wakefulness and quiet sleep led to a progressive disappearance of EMG-Di ($n = 8$) and EMG-CT ($n = 5$) and to an increase in EMG-TA ($n = 7$) during inspiration with an increase in trans-upper airway pressure. Rarely, transmission of NIPPV through the glottis was prevented by complete, active glottal closure during swallows or movements. This phenomenon was most frequent during active sleep epochs, which were characterized by frequent, irregular bursts of TA-EMG. **Conclusion.** Active glottal constriction can occur with high volume NIPPV in non-sedated lambs. We suggest that this is partly responsible for limiting lung ventilation during NIPPV in neonates.

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CYSTIC FIBROSIS AMONG HIGH-RISK POPULATION OF EGYPTIAN CHILDREN- A PILOT STUDY

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Rationale: Cystic (CF) fibrosis is a well-recognized disease in many parts of the world. Little knowledge is available on the incidence and clinical presentation of CF in Egyptian patients. The objective of this study is to screen for cystic fibrosis in a high-risk population of pediatric patients seen at the Cairo University Children's Hospital, Kasr el Aini Faculty of Medicine, Egypt. **Methods:** The study included sixty infants and children 2_ years of age or less who were referred to the pulmonary service at Cairo University Children's hospital, a tertiary care health facility. All recruited patients had one or more of the following: persistent or recurrent respiratory symptoms, failure to thrive, gastrointestinal and hepatic symptoms. Patients were screened for cystic fibrosis using the CF Indicator^{TM} sweat test system (PolyChrome Medical, Inc., Brooklyn Center, MN). This is a qualitative indication of chloride levels in human sweat, positive results indicate sweat chloride >60 mEq/L. **Results:** Of all sixty patients that were screened with the CF Indicator, eleven patients (18%) tested positive for CF. Among CF positive patients, 100% ($n = 11$) showed failure to thrive, 91% ($n = 10$) had chronic or recurrent respiratory symptoms and 64% (7) experienced gastrointestinal symptoms in the form of diarrhea, jaundice and/or liver enlargement. Family history of a similar condition was positive in 27% of CF positive patients while a high incidence of consanguinity (83%) was encountered in all patients studied ($n = 60$). **Conclusion:** The present study suggests that CF is more common in Egypt than previously anticipated. Further nationwide screening studies are necessary to identify the true incidence of CF in the Egyptian population. The clinical manifestations of the disease also appear to differ from the classically described picture of CF, this also warrants further investigation. Finally a comprehensive care program should be established for Egyptian CF patients to prolong survival and improve their quality of life.

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DIAGNOSTIC VALUE OF COMPUTED TOMOGRAPHIC VIRTUAL BRONCHOSCOPY IN PEDIATRIC TRACHEOBRONCHIAL LESIONS

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Virtual endoscopic models can be reconstructed by processing axial computed tomographic (CT) images, this technique is noninvasive and requires minimal patient preparation compared to actual bronchoscopy. **Objective:** To evaluate the diagnostic value and clinical application of CT virtual bronchoscopy (CTVB) in pediatric practice. **Methods:** Twenty-six children with suspected tracheo-bronchial and pulmonary lesions were recruited from outpatient and inpatient departments at the Cairo University Children's Hospital, Cairo, Egypt. During the study period (July 2002–February 2003) all study patients underwent spiral CT of the chest and CTVB as part of their diagnostic work-up. Examination results were compared to findings on actual bronchoscopy or pathologically at the time of surgery. **Results:** Patient age ranged from 2 days–12 years with male to female ratio 1.6:1. Diagnostic categories included tracheo-esophageal fistula (TOF) (27%), external compression lesions (27%), inhaled foreign body (27%), persistent pneumonia (7.6%) and one patient (3.8%) in each of the following groups: tracheo-broncho malacia, tracheal polyps, pulmonary hypoplasia and bronchopleural fistula. Diagnosis was enhanced with CTVB compared to axial CT examination especially in TOF (85.7% v.s. 28.5%) and tracheobronchial foreign body (80% v.s. 40%). Both methods were of equal diagnostic value for compression lesions involving extra bronchial structures e.g. mediastinal lymphoma (100%). Dynamic airway lesions as tracheo-bronchomalacia and mucosal inflammatory changes were not apparent with chest CT nor CTVB but were diagnosed through bronchoscopy which also served to obtain broncho-alveolar lavage samples. Overall, CTVB demonstrated the lesion in 86.3% of cases, while it failed to demonstrate a lesion in 11.5% of cases ($n = 3$) which were later confirmed through bronchoscopy. **Conclusion:** The diagnostic value of axial CT examination for pediatric airway disorders can be extended through the use of CTVB. Diagnostic accuracy increases especially for fixed endobronchial lesions e.g. TOF and inhaled foreign body and for lesions involving structures surrounding the trachobronchial tree. CTVB complements actual bronchoscopy for comprehensive patient evaluation.

EFFECT OF MATURITY ON THE PROLIFERATION AND REACTIVITY OF CULTURED HUMAN AIRWAY SMOOTH MUSCLE CELLS, AND CONTRACTION OF ISOLATED RAT TRACHEAE

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Rationale. Asthma very often begins in childhood, and is characterized by airway inflammation, hyperresponsiveness and obstruction. The airway smooth muscle cell plays a major role in all the above, which lead to airway remodeling. However, the ontogenesis of myocyte responses have been not been extensively studied. The aim of the study was to evaluate 1) human myocyte proliferation and 2) intracellular calcium [Ca^{2+}]i signalling 3) rat

isolated tracheae contraction, in immature and adults tissue. **Material and methods.** Human tissue was obtained (with the parents' informed consent) from neonates who died in the NICU and adults who had undergone lobectomies due to lung cancer. After dissection using a microscope, the cells were cultured, and the presence of pure myocytes confirmed by immunochemistry (anti- α-actine and -myosin antibodies). Cell proliferation was studied by *Thymidine incorporation, and $[Ca^{2+}]i$ by microspectroflurimetry using indo-1 (at baseline, and after stimulation by bradykinin, U46619, histamine and acetylcholine, in the presence or absence of TNFα or IL-6). In 12-day-old and 15-week-old Wistar rats, the tracheae were dissected and cumulative isometric force to the stable muscarinic agonist carbachol measured in standard conditions, in the presence or absence of the proinflammatory cytokines TNFα and IL-1β. **Results.** 1) Neonatal cultured myocytes proliferated approximately 30% faster than adult myocytes 2) $[Ca^{2+}]i$ response in neonatal myocytes was maximal for short incubation times (<3 days) and was more marked than in adults regarding histamine or after TNFα stimulation. In contrast, in adults, responses to bradykinin, and acetylcholine (after cytokine stimulation) were of greater magnitude 3) In the presence of combined TNFα and IL-1β, immature rat airway contractility is more enhanced than in adults. **Conclusion.** Immature human airway myocytes and rat tracheae are in many respects more susceptible to inflammation, and may produce more hyperreactivity (to certain agonists) and remodeling than adults.

PLATELET-ACTIVATING FACTOR RECEPTOR (PAFR) AND RESPIRATORY AND METABOLIC ADAPTATIONS TO HYPOXIA IN TRANSGENIC MICE

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Activation of PAFR leads to quantal release of glutamate at the synaptic cleft, and regulates neural transmission. However, excessive PAFR activation may lead to neuronal excitotoxicity. In previous studies, a presynaptic PAFR blocker reduced the peak hypoxic ventilatory response (pHVR) but not hypercapnic ventilatory responses (HCVR) in rats (Gozal et al., Am J Physiol 1998; 275:R604). To further examine the role of PAFR in respiratory control, PAFR −/− and PAFR +/+ adult male transgenic mice underwent hypoxic (10% O_2 balance N_2 for 20 min) and hypercapnic (5% CO_2 balance air for 20 min) challenges in a whole-body plethysmograph. Metabolic rate was measured by indirect calorimetry. HCVR was similar in the 2 groups (p-NS). However, pHVR was significantly reduced in PAFR −/− mice ($p<0.001$), with reduced tidal volume recruitments during pHVR accounting for the differences between the 2 groups. In addition, hypoxic ventilatory depression was attenuated in PAFR −/− mice ($p<0.01$), and was mediated by attenuation of progressive decreases in oxygen consumption during sustained hypoxia in PAFR −/− mice ($p<0.01$). Based on these findings, we examined the effects of PAFR on intermittent hypoxia (IH) induced long-term ventilatory facilitation. PAFR +/+ and PAFR −/− mice were exposed to IH consisting of 90 sec 21% O_2 and 90 sec 10% O_2 for 30 days and ventilatory recordings were then performed. Normoxic ventilation in PAFR −/− was significantly reduced compared to PAFR +/+ mice ($p<0.01$). Thus, PAFR expression and/or function modulates components of the ventilatory and metabolic adaptations to hypoxia, but not to hypercapnia. We postulate that imbalances in PAFR activity may lead to maladaptive regulation of the tightly controlled relationships between ventilatory and metabolic pathways during hypoxia.

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INTER-OBSERVER AGREEMENT IN THE RADIOLOGICAL DIAGNOSIS OF LOWER RESPIRATORY TRACT INFECTIONS IN CHILDREN

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Objective: To evaluate the inter-observer agreement on the radiographic diagnosis of lower respiratory tract infections in children. **Methods:** Chest X-Rays (CXR) from sixty children younger than 5 years of age were evaluated by three physicians: a pediatric radiologist (PR), a pediatric pulmonologist (PP) and an experienced emergency pediatrician (EP). All children had visited the Emergency Room for having an acute respiratory infection, with apparent lower respiratory tract involvement. Observers were blinded for the original diagnostic conclusions, but clinical and laboratory data from the initial medical evaluation were handed-in with each film. **Variables:** Grouped in five categories: a) film quality; b) site of abnormality; c) radiographic patterns; d) other radiographic images; e) diagnosis. Statistics: Inter-observer agreement was assessed using Kappa statistics, accepting its prevalence-bias-adjusted values (PABAK). **Results:** Kappa values for each of the three observers pairs formed (RP x PP, RP x EP, and PP x PE) were: 0,41, 0,43, and 0,39 respectively. The overall inter-observer agreement was moderate (0.41). Agreement on other variables: regular on ''technical quality'' (0.30); moderate on ''site of abnormality'' (0.48); fair on ''radiographic patterns'' (0.29); moderate on ''other radiographic images'' (0,43); and moderate on ''diagnosis'' (0.33). The overall intra-observer agreement was ''moderate'' (0.54), although lower than the ones reported in the medical literature on CXR variability. **Conclusions:** Inter-observer variability is an intrinsic characteristic of CXR interpretation, and to ascertain the exact diagnosis of LRTI in children has its challenges. No similar study had been done in Brazil, however, our results were similar to those reported on different medical journals.

Key words: Lower respiratory infections, pneumonia, diagnosis, chest x-ray.

TRANSFORMING GROWTH FACTOR (TGF)-BETA, INDUCED FIBROBLAST (FB) PROLIFERATION AND ALPHA-SMOOTH MUSCLE ACTIN (ALPHA-SMA) EXPRESSION ARE EFFECTIVELY INHIBITED BY FLUTICASONE PROPIONATE (FP) AND SALMETEROL (SAL) (SFCT03)

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ItalyAirway remodelling in asthma is associated with the presence of an increased numbers of activated Fbs, a great proportion of which express α-SMA, i.e. have the phenotypic characteristics of myofibroblasts (MyoFbs). Experimental models suggest that MyoFbs in the airways might be the primary source of type I collagen gene expression at sites of active fibrotic processes. This study evaluated whether i) TGF-β, a cytokine secreted by inflammatory cells in chronic airway disorders, could induce Fb proliferation and MyoFb differentiation and ii) two drugs prescribed in the treatment of obstructive airway diseases, i.e. FP and/or SAL, could modulate these Fbs functions. Primary human airway Fb cultures were stimulated with TGF-β in the presence of FP+/−-SAL (10 and 100 nM); cell proliferation (Prol) was detected by ^3H thymidine incorporation as count per minute (cpm), α-SMA expression (exp) was evaluated by immunocytochemistry as % of α-SMA positive cells and the effect of the drugs was reported as % inhibition of the TGF-β-stimulated Fb function. TGF-β was able to induce an increase in cell Prol (Ctr:1494 ± 190.5 cpm, TGF-β: 4049 ± 643.8 cpm, $p = 0.001$) and in α-SMA exp (Ctr: $7.4 \pm 1.1\%$; TGFb: $18.7 \pm 3.7\%$, $p = 0.002$). Cell Prol was significantly reduced by FP (10nM: $81.0 \pm 1.1\%$, 100nM: $73.3 \pm 5.9\%$), SAL (10nM: $45.1 \pm 7.3\%$ 100nM: $38.1 \pm 8.3\%$), or by FP + SAL combination (10nM: $86.8 \pm 1.7\%$, 100nM: $83.5 \pm 2.8\%$) ($p<0.05$). A significant inhibition of the upregulation of α-SMA exp induced by TGF-β was also observed in the presence of FP (10nM: $53.0 \pm 11.4\%$, 100 nM: 50.0 ± 7.7), SAL (10nM: $40.8 \pm 11.2\%$, 100nM: $15.6 \pm 16.7\%$) or the combination FP + SAL (10nM: $49.6 \pm 13.0\%$, 100nM: $64.4 \pm 8.0\%$), ($p<0.05$). The combination of the two drugs was more effective than the two drugs alone in inhibiting cell Prol but not α-SMA exp. Thus, in addition to corticosteroids, also long-acting-β_2-

agonists are able to downregulate *in vitro* fibroblast functions, likely involved in airway remodelling.

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ATOPY AND ALLERGIC RESPIRATORY DISEASE IN RURAL POLAND; THE EFFECTS OF FARMING AND NONFARMING CHILDHOODS

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Objectives: to measure the prevalence of atopy and allergic respiratory disease in families living in two rural comunities in south-west Poland; and to examine the relationship between atopy or asthma and a rural farming/ non-farming childhood. **Methods:** a cross-sectional survey of a representative population aged 5 years and over living in five villages and a small, nearby town. A questionnaire enquiring into symptoms of allergic disease and relevant environmental exposures was completed and atopy determined by responses to skin prick tests with four common aeroallergens (mixed grass pollens, house dust mite, cat, mixed tree pollens). Response rates were high (86% in the villages, 91% in the town). Overall, 1715 persons—1347 adults and 368 children—in 501 families were surveyed. **Results:** just 4% of those living in the villages had a positive response to one or more skin prick tests - in most cases to house dust mite. Very few villagers had positive tests to cat fur or pollens. Atopy was much more common (11%) among town inhabitans; in this case, grass pollen produced most positive responses. Current asthma, diagnosed by a doctor, was present in 4% of those who had been brought up on a farm—a figure very similar to that among those who had not (5%). Seasonal allergic rhinitis (pollinosis) based on the doctor's diagnosis was more common in town inhabitants : 7% vs. 3%. **Conclusions:** Farm environment in childhood seems to have a protective effect against seasonal allergic rhinitis diagnosed and aeroallergen sensitization but not for asthma diagnosed by a doctor.

GENE EXPRESION PATTERNS IN PERIPHERAL BLOOD OF SNORING CHILDREN

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Introduction: Obstructive sleep apnea syndrome (OSA) is characterized by episodic hypoxemia, hypercapnia, and arousal during sleep, and is associated with significant cardiovascular and neurocognitive deficits in children. Gene expression studies show that the expression of selective clusters of genes changes in response to intermittent hypoxia as well as to transition from sleep to wakefulness and sleep deprivation. **Hypothesis:** Sleep-associated alterations in OSA will lead to a unique pattern of gene expression changes in the blood of children with either primary snoring (PS) or OSA . **Methods:** Peripheral blood samples were drawn immediately after sleep from snoring children and controls. OSA was defined as AHI≥5, PS as 1<AHI<5, and Controls as AHI<1. Purified mRNA was used as template for double stranded DNA synthesis, and c-RNA transcription. Labeled probe was hybridized with Affyemtrix Human Genome U133A oligonucletoide microarrays that allow the simultaneous profiling of ~20,000 genes. For data clustering and visualization we used publicly available software packages such as Treeview, GeneCluster, and Click. For statistical analysis we used non-parametric scoring methods, TNoM and Info available in Scoregene Package. **Results:** Of the ~20,000 represented on the array, expression levels for 194 genes were significantly higher and 178 genes were lower in the OSAS group compared to the non-OSAS group. This was higher (double) than predicted by the *null* hypothesis. Subset gene analyses permitted identification of multiple regulated genes that did not overlap between OSA and non-OSA, with major differentially-regulated gene categories encompassed among cell survival, cytoarchitectural integrity and apoptosis, metabolism, and inflammation-related genes. Furthermore, cluster analysis predicted accurate identification of all OSA patients with a sensitivity of 100% and a specificity of 75%. **Conclusions:** OSA children exhibit distinct gene expression patterns in their peripheral blood, and such patterns allow for accurate identification of their condition. Further prospective large-scale studies to validate this approach and potential implications are needed.

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EARLY CAT-OWNERSHIP IS ASSOCIATED WITH LOWER PREVALENCE OF INHALANT ALLERGEN (INCLUDING CAT DANDERS) SENSITIZATION AND ATOPY-RELATED RESPIRATORY SYMPTOMS

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The role of cat ownership in the development of allergic sensitization and respiratory symptoms (ResSym) has been studied with different results, possibly because of confounding genetic or environmental factors. The aim of the study was to evaluate whether in a Mediterranean region (Liguria), the prevalence of: a) allergic sensitization, and specifically cat sensitization, and b) ResSym (asthma and/or rhinitis related symptoms) could be influenced by cat-ownership. A group (gr) of 644 children [6 (4-9) yrs old, male-to-female ratio: 1.34] were consecutively enrolled: 307 had never kept cats at home (No-cat gr: 47.7%) and 337 are/were cat-owners (Cat gr: 52.3%), 95% of which had only one cat. The Cat gr was divided into 2 subgrs: A) cat at home in the first 2 yrs of life and B) cat at home later in life. Sensitization to cat and/or to other inhalant allergens by skin prick test and ResSym were evaluated. 342 children (53.1%) were sensitized to allergens, including 76 (22.2%) who were sensitized to cat. The proportion of sensitized children was significantly lower in the Cat gr than that in the No-cat gr (49% vs 57.7%, $\chi^2 = 4.9$, $p = 0.03$) even though no significant difference was found in the frequency of sensitization to cat ($p = 0.1$) between the 2 grs. Moreover, the Cat gr had less frequently ResSym than the No-cat gr (74.5% vs 81.8%, $\chi^2 = 5.0$, $p = 0.03$). Evaluating the 2 grs of cat owners, we found that the proportion of sensitized children and the frequency of sensitization to cat were significantly lower in Cat gr A than in Cat gr B (44% vs 63.2 %, $\chi^2 = 9.7$, $p = 0.01$ and 7.9% vs 15.3%, $\_2 = 3.9$, $p = 0.048$, respectively). In addition, Cat gr A had less frequently ResSym than Cat gr B (71% vs 84.7%, $\chi^2 = 6.3$, $p = 0.01$). No differences within grs and subgrs were seen between children with or without family history of atopy ($p > 0.1$). Thus, early cat-ownership was related to a lower prevalence of sensitization to aeroallergens (including cat danders) and of asthma/ rhinitis-related symptoms.