251. Cell biology, blood and sputum biomarkers in airway diseases

P2220

A novel, simple and rapid method to measure soluble mediators in dithiothreitol-treated sputum

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Sputum is most often processed by the addition of dithiothreitol (DTT) to liquefy the sputum plug and release cells and soluble mediators for subsequent analysis, but DTT can interfere substantially with these analyses. The aim of this study was to develop a method to reverse this, to allow for the sensitive determination of analytes in DTT treated sputum by the addition of the thiol oxidising agent diamide. This was applied to the immunoassay of a panel of 21 analytes.
Assays of these analytes were shown to be suppressed to a highly variable degree (0-900-fold, median 25-fold) by the presence of DTT, as a result of a worsening of both background and maximal signal. Treatment of DTT-containing matrix with diamide resulted in a substantial improvement in the signal to background ratio, representing 1-150 fold improvement (median 5-fold) in the assay. This diamide method was applied to the assay of analytes in the presence of DTT and permitted the sensitive (limit of detection (LOD) <10pg/ml) assay for all analytes except MDC (LOD 136pg/ml), with a high signal to background ratio (median 3700 at 10ng/ml) and acceptable recoveries of spiked sputum supernatants (median 72%). This protocol was applied to DTT sputum obtained in a study of the dose- and time-dependence of responses to LPS inhalation in healthy smokers as a model of COPD bacterial exacerbations (Aul et al 2012, Brit J Clin Pharmacol in press). We were able to demonstrate significant increases in IL-1β, TNFα, MCP-1 and MIP-1β in sputum, with a maximal effect at 6h.
This simple and rapid diamide method therefore provides a novel way to treat DDT sputum to allow for the sensitive immunoassay of a wide range of analytes.

P2221

C-reactive protein levels in COPD according to clinical parameters, smoking behavior and pulmonary hypertension

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Aim: To assess relationship between serum CRP levels and clinical parameters known to predict outcome, smoking history and biomass exposure in COPD.
Methods: Spirometry, echocardiography, Sp02 measurements and serum CRP levels were assessed in 89 stable COPD patients and 60 age- and sex-matched healthy subjects. BODE index scores were assessed in COPD patients. Associations between CRP levels and clinical parameters were evaluated.
Results: Of the COPD group (11% stage 1, 48% stage 2, 29% stage 3, 11% stage 4) mean age is 60,6±8,5 years. CRP levels are higher in COPD patients than in controls (7.22±9.84 mg/L, 3.14±2.27 mg/L; p=0.005). CRP levels were not significantly different between COPD patients treated with inhaled corticosteroids and those not treated (7.90±10.65 mg/L, 6.17±8.46 mg/L; p>0.05). Significant relationship is found between CRP levels and FEV1, FEV1%, FVC, FVC%, SpO2, MMRC dyspnea scale, 6 minute walk distance and BODE scores. Using multivariate analysis BODE scores and coexistence of systemic hypertension manifested the strongest association. CRP levels in COPD patients with and without pulmonary hypertension were significantly different (11.86±13.38 mg/L, 5.78±8.05 mg/L; p=0.012). CRP levels did not differ significantly according to smoking status and biomass exposure in COPD patients though COPD cases due to biomass exposure who never smoked also had higher CRP levels compared to healthy controls (9.16±10.03 mg/L, 3.14±2.27 mg/L; p=0.028).
Conclusion: Systemic inflammation is related to disease severity and to concomitant systemic hypertension and pulmonary hypertension in COPD patients independent of smoking status or biomass exposure.

P2223

Serological detection of elastin fragments in COPD and IPF patients

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Introduction: Elastin plays a critical role in the development of respiratory system disorders including COPD and IPF, whose pathogenesis involves an inflammatory response and tissue turnover mediated by proteases, especially matrix metalloprotease (MMP)-12 secreted by activated macrophages.
Aims and objectives: Our aim was to evaluate whether a novel biochemical marker of elastin degradation mediated by MMP-12 may provide information in relation to lung tissue destruction during pulmonary disease and aid in the diagnosis of respiratory disease.
Methods: Human elastin was in vitro cleaved by different proteases and the resulting peptides were analyzed by LC-MS/MS. Among more than 400 fragments, the MMP-12 generated elastin neoepitope cleaved at the amino acid position 444 (ELN-441/ELM) was chosen as candidate for antibody generation for its uniqueness for human elastin following assay development. This novel marker was assessed in serum collected in a small cohort of COPD (n=10), IPF (n=29) patients and controls (n=11) using a competitive enzyme linked immunosorbent assay (ELISA).
Results: Serum levels of ELM were significantly higher in patients diagnosed with COPD (p<0.0003) and with IPF (p<0.0001) compared to controls. The diagnostic value, measured by means of the area under the curve of receiver operating characteristic (AUROC) was best in COPD patients (AUROC 97%, p=0.00025) and lower but still significant in IPF patients (AUROC 90%, p=0.00011).
Discussion: Even though these findings need to be validated in larger clinical settings, the ELM marker described showed potential for the separation of controls from COPD or IPF patients.

P2224

Laryngopharyngeal pepsin reflux in chest clinic patients with upper airways symptoms

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Introduction: Laryngopharyngeal reflux (LPR) may underlie both chronic cough and vocal cord dysfunction, and one explanation for a lack of response to standard anti-reflux therapy may be the persistence of non-acid-reflux. Salivary pepsin is a potential biomarker for LPR. The aim of this study was to evaluate if the detection of pepsin in saliva was associated with signs of laryngopharyngeal reflux in patients having nasendoscopy for investigation of upper airway symptoms.
Methods: We recruited patients from the Airways Clinic requiring nasendoscopy. All patients had the Reflux Finding Score (RFS) recorded at nasendoscopy. Salivary pepsin was quantified with a lateral flow device using monoclonal antibody labelling (Peptest, RD Biomed, UK).
Results: Of 20 patients recruited, 12 were confirmed to have VCD and 13 a clinical suspicion of LPR (based on an RFS > 7). Pepsin was detected in the saliva of 11/20 subjects (55%), including 67% of the VCD patients and 61% of those with a high RFS, although 43% of those with a low RFS also had a positive pepsin. Salivary pepsin had a sensitivity of 62% and specificity of 57% for predicting a high RFS. There was no significant correlation between RFS scores and salivary pepsin. Seven of the 10 patients already on treatment for a clinical diagnosis of reflux had a positive pepsin assay.
Conclusions: Salivary pepsin was frequently present in patients with upper airway symptoms, and not strongly related to clinical findings of reflux, suggesting a high prevalence of sub-clinical LPR. Further investigation should determine the clinical relevance of this, and whether LPR treatment results in an improvement in pepsin levels and the associated upper airway symptoms.

P2225

Comparison of serum osteopontin levels in patients with exacerbations and stable chronic obstructive pulmonary disease

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Background: Osteopontin is reconized as an important adhesive bone matrix and a key cytokine involved in immune cell recruitment, tissue repair and remodeling. Then serum levels of osteopontin have not been evaluated in patients with chronic obstructive pulmonary disease(COPD). The aim of this study is to evaluate and compare the serum levels of osteopontin in patients with exacerbations and stable COPD
Methods: Serum samples were obtained from 22 healthy control subjects, 18 stable COPD patients, and 15 COPD with exacerbation patients. Serum concentrations of ostepontin were measured by the ELISA metnod.
Results: Serum levels of osteopontin were higher in patients with exacerbation than with stable COPD and in healthy control(62.4±51.9 ng/mL, 36.9±11.1 ng/mL,30.0±11.0 ng/mL, p=0.003). Osteopontin levels were significantly decreased after clinical improvement than during exacerbation (45±52.1 ng/mL, 62.4±51.9 ng/mL, p=0.160). Also osteopontin levels showed a significantly negative correlation with forced expiration volume in one second(FEV1%) in healthy controls and stable COPD (r= -0.389, p=0.013). C-reactive protein was positively correlated with osteopontin levels in patients with COPD exacerbations(r=0.775, p=0.002).
Conclusions: The serum levels of osteopontin were increased in patients with COPD exacerbations and tended to decrease after clinical improvement. These results suggest the possible role of osteopontin as a biomarker of COPD with exacerbation.

P2226

Correlation between sputum cytology and lung physiology in asthma and COPD subjects in Indian population

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Airway cellular inflammation is known to contribute to the pathophysiological consequences of asthma and COPD; however, the differences in their profile and association with lung function parameters have not been investigated in Indian population. We aimed to investigate the relationship between inflammatory cellular profile and lung functions in asthma and COPD.
Methods: 25 asthma & 29 COPD patients underwent pre/post spirometry in accordance to ATS/ERS criteria & sputum induction with 3% hypertonic saline, processed with 0.1%DTT to investigate cellular inflammatory profile in their airways.
Results: A significant difference was observed between COPD & asthma in total median neutrophil counts [113.6 (IQR:65.4, 161.8) x106/g vs 5.9 (IQR:0.1, 30.2) x106/g, p=<0.0001)] & lymphocytes [8.5 (IQR:0.2, 84.0) x106/g vs 1.2 (IQR:0.2, 4.4) x106/g, p=0.02)]. There were no significant difference between the 2 groups in absolute eosinophil count (p=0.12). However, the mean eosinophil was 23.3% ± 24 in asthma & 7% ± 8.5 in COPD (p=0.006). There was a negative correlation between the % neutrophils and FVC in asthma (r=-0.478, p=0.02), while in COPD there was a negative correlation between % eosinophils & FVC% (r=-0.380, p=0.05) & between absolute eosinophil count and FEV1% (r=-0.380, p=0.05). There was a positive correlation between absolute eosinophil count and duration of COPD (r=0.391, p=0.04) and between absolute macrophage count and FEF25-75/FVC in COPD (r=0.490, p=0.01).
Conclusion: This study showed that presence of neutrophils were related to greater loss of lung volumes in asthma while presence of eosinophils was related to COPD disease severity and duration among Indian subjects.

P2227

Validity of serum surfactant protein D as a lung-specific biomarker in monitoring asthma, chronic obstructive pulmonary disease and chronic bronchitis in Lebanese patients: Preliminary results

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Surfactant protein D (SP-D) is an oligomeric lung-derived lectin with important roles in innate host defence. Previous studies have suggested serum SP-D as a reliable biomarker for inflammatory lung diseases.
Our objective was to validate for the first time in the Lebanese population, SP-D as a clinical biomarker for asthma, COPD and chronic bronchitis. In addition, we studied the correlation between SP-D level and the disease severity. A case-control study is being conducted in the Lebanese population. To date, 46 cases of asthma, 24 cases of COPD, 23 cases of chronic bronchitis patients and 46 controls have been analysed. Lung function measurements were performed by spirometry, a standardized questionnaire was filled and serum levels of SP-D, CRP and plasma fibrinogen levels were measured.
The mean level of SP-D was significantly elevated in asthmatics (1394.26 $ng.ml^{-1}$; p = 0.023), in COPD patients (1507.24 $ng.ml^{-1}$; p = 0.013) and in chronic bronchitis patients (1477.27 $ng.ml^{-1}$; p = 0.007) when compared to controls (980.1 $ng.ml^{-1}$). Serum SP-D levels correlated significantly with the disease severity only in COPD (p = 0.007). Conversely, levels of CRP and fibrinogen did not correlate with disease severity.
We conclude that SP-D could be used as a biomarker for asthma, COPD and chronic bronchitis and seems to be more specific than general systemic markers of inflammation, CRP and fibrinogen. The ongoing study should provide further information on the cut off values and/or absolute values of serum Sp-D reflecting the degree of inflammation in the investigated diseases.

P2228

Systemic inflammation in older adults with asthma-COPD overlap syndrome

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Background: The overlap of asthma and COPD is common in older people with obstructive airway disease (OAD). Systemic inflammation is associated with adverse clinical outcomes and co-morbidities in COPD, but its role on asthma-COPD overlap syndrome is unknown. This study aimed to examine systemic inflammation in asthma-COPD overlap, and the potential clinical relevance with OAD.
Method: Serum high sensitivity C-reactive protein (hsCRP), Interleukin 6 (IL-6) and serum amyloid A (SAA) were measured in 108 adults older than 55 years comprising healthy controls (n=27), asthma (n=7), COPD (n=29) and asthma-COPD overlap (n=45). Spirometry, induced sputum, quality of life, co-morbidities and medications were assessed. Levels of systemic inflammatory mediators were compared, and the associations with clinical characteristics were tested in multivariate regression model.
Results: Patients with asthma-COPD overlap had significantly elevated IL-6 levels. SAA level was raised in both the COPD and asthma-COPD overlap groups. CRP level was significantly increased in COPD. The disease groups had different patterns of systemic inflammation. CRP was positively associated with BMI, whereas IL-6 was predicted by age, FEV1%predicted, and cardiovascular disease. SAA level was associated with co-morbidity, and females had higher SAA level than males. Systemic markers were not associated with airway inflammation.
Conclusion: Systemic inflammation is a common and important component of the asthma-COPD overlap. The pattern of systemic inflammation in asthma-CDPD overlap is differing from COPD characterized as an elevated IL-6 and SAA levels. It is not related to airway inflammation, and may be an independent treatment target.

P2229

Repeatability and inter-relationships of small airway biomarkers in asthma

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Background: There is evidence that small airway dysfunction may have an important role in asthma. We aimed to determine the within-visit and between-visit repeatability of small airway biomarkers in asthma, and explore the inter-relationships between them and with standard pulmonary function tests.
Methods: We recruited ninety-eight patients with asthma. All participants attended a baseline visit at which they undertook spirometry, body plethysmography, measurement of carbon monoxide diffusing capacity, impulse oscillometry (IOS), multiple breath washout (MBW) and induced sputum cell count. Eighteen patients undertook two-week and three-month follow-up visits, and twenty-six patients undertook six-month follow-up visits, at which all physiological tests were repeated.
Results: Small airway biomarkers displayed excellent within-visit repeatability (intraclass correlation coefficient [ICC] > 0.9), with the exception of S_{cond}, which was only moderately repeatable (ICC = 0.629). The biomarkers were all very stable over a three-month time frame, but S_{cond} and S_{acin} were only moderately stable over six months. Principal components analysis of the variables derived five components, corresponding to the concepts of:
1) Expiratory flow limitation/air trapping
2) Heterogeneous airway constriction/closure
3) Ventilation heterogeneity
4) Airway inflammation
5) Impaired diffusion capacity
Conclusions: Small airway biomarkers are repeatable and stable over three months. Moreover, they appear to provide additional and independent physiological information over and above standard pulmonary function tests.

P2230

Metabolic syndrome and chronic diseases in COPD

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Introduction: In this study, we examined accompanying comorbidities and metabolic syndrome (MS) in patints with COPD
Method: Sixty-six patients with COPD and 40 subjects as control group were included. The index severity of chronic diseases was evauated by using the MCIRS. MS was defined according to National Cholesterol Education Program.
Results: MCIRS was significantly greater in patients with COPD than in control group. MS was detected in 18 patients (27.3%) in patients with COPD and in 8 patients (20%) in control group. The rate of MS in patients with stage II COPD was higher than in in patients with stage IV COPD (p: 0.04).

Table 1. The Comparison of the Rate Disorders of Metabolic Syndrome according to Stage of COPD

	Stage I COPD	Stage II COPD	Stage III COPD	Stage IV COPD
The rate of Metabolic Syndrome	0/3	13/33*	4/15	1/10
The rate of Metabolic Syndrome	0%	33.3%	26.5%	10%

*The presence of statistical significance difference acccording to the stage IV COPD.

The MCIRS showed inverse associations with FEV1%, FVC%, FEV1/FVC% while positive association with BODE index.
Conclusion: Comorbidities and MS are diseases which are seen in different form's of COPD.

P2231

Microalbuminuria in chronic obstructive pulmonary disease

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Introduction: We investigated levels of microalbuminuria and the factors determining in patient with COPD
Method: 66 patients with COPD and 40 subjects as control group were included. The presence of microalbuminuria (MAB) was defined when the urinary albumin creatinin ratio (UACR) was between 20 mg/g in men and 30 m/g in women.
Results: Pearson correlation showed that there were inverse associations between UACR and PO2, FEV1%, FVC% and positive relation between UACR and BODE index. In linear regression model, the UARC showed inverse association with PO2 while positive association with MRCI, and BODE index.

Table 1. The predictors of urinary albumin/kreatinin ratio in all subjects

	Urinary albumin/kreatinin			Urinary albumin/kreatinin			Urinary albumin/kreatinin	
	B values	p values		B values	p values		B values	p values
Age	–0.10	0.4	Age	–0.18	0.17	Age	0.01	0.9
Sex	0.17	0.1	Sex	–0.01	0.9	Sex	0.05	0.06
BMI	–0.02	0.8	BMI	0.22	0.07	BMI	–0.06	0.6
MRCI	0.31	0.007	MRCI	0.04	0.04	MRCI	0.19	0.1
PO2	–0.22	0.04	FEV1%	–0.12	0.3	BODE index	0.28	0.01

Statistical significance p<0.05. BMI: Body Mass Index, MRCIS: Modified Cumulative Illness Rating Scale.

In logistic regression model, the presence of MAB showed associations with severity of COPD, PO2, BODE index, PO2 and age.

Table 2. The predictors of presence of MAB in all subjects

	Presence of MAB p values		Presence of MAB p values		Presence of MAB p values
Age	0.003	Age	0.02	Age	0.007
Sex	0.4	Sex	0.3	Sex	0.3
Smoke p/y	0.7	Smoke p/y	0.9	Smoke p/y	0.7
Severity of COPD	0.04	BODE index	0.02	PO2	0.003

Statistical significance p<0.05. BMI: Body mass index, COPD: Chronic obstructive pulmonary disease.

Conclusion: Microalbuminüria may be seen in patints with COPD depending on severity of disease and hypoxemia.

P2232

Chronic mucus hypersecretion in asthma: Relation to smoking status and disease severity

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Background: Chronic mucus hypersecretion occurs in chronic asthma, but the effects of smoking status and disease severity on this symptom are not clearly established. We assessed the prevalence of chronic mucus hypersecretion in patients with asthma recruited to the Glasgow COPD and Asthma Biomarker study.
Methods: One hundred and twenty subjects with asthma, smokers and never smokers of different GINA severity and fifty-four COPD subjects of different GOLD severity were recruited. Assessments included demographics, history of chronic cough and sputum production and spirometry.
Results: Baseline demographic and lung function data in the smokers with asthma and never smokers with asthma were similar. The COPD group were older and had a higher pack year history than the asthmatic group. Chronic mucus hypersecretion was strongly associated with smoking in the whole asthma group (Chi-sq=22.8, p<0.001). The proportion of patients with asthma and COPD of different disease severity and smoking status giving a history of chronic mucus hypersecretion is shown in Table 1.

Table 1

	Mild	Moderate	Severe	p*
Smokers with asthma (n=61)	10/20 (50.0%)	6/18 (33.3%)	18/23 (78.3%)	0.012
Never smokers with asthma (n=59)	1/19 (05.3%)	3/18 (16.7%)	8/22 (36.4%)	0.045
COPD (n=54)	8/14 (57.1%)	15/23 (65.2%)	8/16 (50.0%)	0.641

*Fisher's Exact test.

Chronic mucus hypersecretion increased with asthma severity overall (p=0.003) and in the smokers and never smokers with asthma, but not in COPD.
Conclusions: Chronic mucus hypersecretion is a common symptom in adults with chronic asthma particularly in smokers and patients with severe disease. This finding suggests that novel treatments targeting this symptom may be of value in the management of asthma.

Abstract P2230 – Table 2. The predictors of MCIRS in all subjects

	MCIRS			MCIRS			MCIRS			MCIRS			MCIRS	
	B values	p values		B values	p values		B values	p values		B values	p values		B values	p values
Age	0.46	0.0001	Age	0.31	0.0001	Age	0.40	0.0001	Age	0.38	0.0001	Age	0.44	0.0001
Sex	–0.16	0.08	Sex	–0.19	0.02	Sex	–0.19	0.02	Sex	–0.18	0.03	Sex	–0.25	0.006
BMI	–0.02	0.7	BMI	– 0.06	0.4	BMI	–0.10	0.2	BMI	–0.13	0.1	BMI	–0.07	0.4
PO2	–0.11	0.3	BODE index	0.34	0.0001	FEV1%	–0.31	0.0001	FVC%	–0.25	0.005	FEV1/FVC%	–0.20	0.02

Statistical significance p<0.05. BMI: Body mass index, MCIRS: Modified Cumulative Illness Rating Scale.

P2233

The role of regional distribution (RD) of body fat mass (BFM) in fat-bone interactions in men with chronic obstructive pulmonary disease (COPD)

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The aim: Evaluation of the relationships between RD of BFM and TNF-α, some hormones and markers of bone metabolism.

Methods: We used DEXA to analyze RD of BFM. We determined: serum leptin (L), testosterone free (TF), TNF-α, parathyroid hormone (PTH), beta-crosslaps (beta-CTX) and osteocalcin. We examined 83 COPD pts (40-70 yrs old) and control group – 15 healthy comparable men. The COPD pts were subdivided into 3 groups (GOLD).

Results: Fat mass (FM) in Android region (A) positively correlated with TNF-α (r=0.33, p=0.02) in all pts group. Trunk and Arms FM were positively related to L (r=0.36 and r=0.35; respectively) in all pts group. L level exerted negative influence on bone mineral density (BMD) in lumbar spine (r=-0.43, p=0.001), but not at femoral necks in total group. We found the positive correlations between beta-CTX and total FM (r=0.33) and FM in A (r=0.32) in all pts group. The total, arms, legs and trunk FM, FM in Android and Gynoid (G) regions were increased in pts with a lower level of TF ($p<0.05$). Loss of BMD positively correlated ($p<0.05$) to loss of BMI, A/G ratio, FM in all body regions, PTH, calcium ionized and was inversely related with L level in all pts group. Although these pts had decrease of BMD, BMI was increased (29.4 in pts with osteopenia and 24.7 kg/m^2 – with osteoporosis). Lowering of BMD might result from other factors, such as TNF-α and some hormones which are produced by the adipose tissue.

Conclusion: The study of RD of adipose tissue in the patient's body is more informative than BMI for assessing the likelihood of developing osteoporosis in men with COPD.

P2234

The indices of body composition (IBC) consideration the changes of serum TNF-α, hormones and oxygen saturation (SO2%) in men with chronic obstructive pulmonary disease (COPD)

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Aim and objectives: to analyze dynamics of changes of IBC and the relationships between IBC and TNF-α, leptin (L), parathyroid hormone (PTH), testosterone free(TF), markers of bone metabolism, SO2%.

Material and methods: Bone mineral content (BMC), Fat mass (FM) and Lean mass (FFM, excl. BMC) were detected by DEXA. We obtain FMI, FFMI and BMCI indices. We examined 83 male pts (aged 40-70) and control group: 15 healthy comparable men. The pts were subdivided into 3 groups according to severity (GOLD).

Results: The value of FFMI was significantly decreased in the 3rd group (17.7 kg/m^2) vs. the control (20.3), the 1st and 2nd groups (20.5 and 20.2 respectively). The significant positive correlations were detected between FMI and L, beta-crosslaps levels and negative one – with TF (r=0.31, r=0.31, r=-0.38 respectively). The TF level was lower ($p< 0.05$) in all pts group vs. the control (6.7 and 10.5 pg/ml respectively). The TNF-α level was inversely related to FFMI (r= - 0.30, p=0.03). BMCI was significantly and positive correlated with SO2% (r=0.40) and the level of PTH (r=0.54). FMI, FFMI and BMCI were correlated with pack/years index (r=- 0.37; r=- 0.37 and r=- 0.30 respectively). Pts of the 3rd group had lower FMI (4.68 kg/m^2) than pts with early COPD stages (8.28 and 9.72 kg/m^2 in the 1st and 2nd groups; $p< 0{,}001$). The BMCI level in 1st and control groups was the same and was significantly higher (1.06 kg/m^2) vs. the 2nd and 3rd groups (1.00 and 0.89 kg/m^2 respectively).

Conclusions: The dynamic of IBC in these pts depends on levels of severity of systemic inflammation, hypoxia, and hormonal changes.

P2235

WITHDRAWN

P2236

Enzymatic activity and clinical parameters in non-cystic fibrosis bronchiectasis: A cohort analysis

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Introduction: Non-cystic fibrosis bronchiectasis (NCFB) is characterized by a vicious cycle of infection, inflammation and enzymatic actions, reinforcing each other and leading to progressive lung damage. We aimed to investigate the enzymatic activity in a NCFB population, to compare with controls and to correlate with clinical parameters.

Methods: 63 patients (27male, 59±18y) with NCFB were recruited and 16 controls (7male, 56±18y), perfectly matched. Each patient was evaluated by spirometry, Leicester Cough Questionnaire (LCQ) and Sputum Colour Chart (SCC). Sputum was induced with hypertonic saline inhalation and succeeded in 49 NCFB patients and 12 controls. Total/differential cell count in sputum was assessed and total gelatinolytic activity (TGA), neutrophil elastase (NSE) and MMP-9 were measured.

Results: TGA and NSE were higher in patients vs controls (p=0.04 and p=0.0003) and both correlated with neutrophil count (NSE: p=0.009; r=0.38 and TGA: $p<0.0001$; r=0.60). Subanalysis of high value TGA showed that NSE accounted for 82% of the activity vs 18% MMP-9 ($p<0.0001$). There was an inverse correlation between neutrophils and FVC% (p=0.02; r=-0.35) and NSE and FVC% (p=0.04; r=-0.29) in NCFB. No relationship was seen between total LCQ score, LCQ subscores and enzymatic gelatinolytic activity. There was however a significant relationship between the SCC and TGA (p=0.003) and NSE (p=0.01). SCC also correlated with neutrophils (p=0.001).

Conclusion: TGA is significantly higher in NCFB and correlates with indices of inflammation and infection (neutrophils and SCC). The majority of TGA was exercised by NSE (82%) in NCFB. No correlation was seen with radiologic score or LCQ.

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Asthma phenotyping: Correlation between inflammatory phenotype, clinical parameters and associated comorbidities

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Introduction: Cluster analysis revealed 5 clinically different clusters of asthma: mild atopic (1), mild to moderate atopic(2), late-onset non-atopic (3), severe atopic (4) and severe with fixed airflow obstruction (5) (Moore et al, 2009).

Aim: To link differential cell count and associated comorbidities (BMI, smoking, atopy, reflux, sinusitis and bronchiectasis) to 5 clusters of asthma based on clinical features (baseline FEV1, maximal FEV1 and age of onset).

Methods: We retrospectively evaluated clinical records from 140 asthma patients (44% male; 43y ± 14) with induced sputa, recruited from outpatient clinic of the University Hospital Leuven between January 1st 2008 and November 1st 2011 and were free of exacerbation for three months prior to sputum induction.

Results: Cluster 1 accounted for 37%, cluster 2 for 26%, cluster 3 for 14% and cluster 4 for 12%. Cluster 5 was too small for further analysis. Cluster 4 as defined by Moore and coworkers is significantly associated with more neutrophils (median: 72%, interquartile: 60-82%); $p<0.02$) in induced sputum as compared to the other clusters. Relative number of patients with reflux was highest in cluster 3 (55%) compared to cluster 1, 2 and 4 (29%, 34% and 38%). Presence of sinusitis was equally distributed between all clusters (p=0.95).

Conclusion: Severe atopic asthmatics have a predominant neutrophilic airway inflammation. Patients with late-onset non-atopic asthma have the highest rate of reflux. Previously unrecognized bronchiectasis were detected in 9% of patients.

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Human neutrophil peptides as biotracers of functional impairment in COPD smokers

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Background: Human Neutrophil Peptides (HNP) are small molecular weight peptides abundant in neutrophils, which may be released upon activation in the extracellular environment. Nearly absent in the normal lung, HNP have been found increased in airways of patients with neutrophil related inflammatory lung diseases. Recently the presence of HNP has been demonstrated in the lung of current smokers.
Aim: We aimed to assess whether sputum HNP concentrations could be used to trace pulmonary functional impairment during COPD clinical course.
Methods: A consecutive population of 138 smokers with and without COPD was referred to our smoking cessation centre. All participants underwent physical examination, pulmonary function tests; sputum samples were collected at enrolment.
Results: We evaluated 42 symptomatic smokers; 8 individuals with COPD stage 1; 18 subjects with COPD stage 2; 19 individuals with stage 3 disease and 51 patients with COPD stage 4. The HNP sputum concentrations were significantly higher in participants with COPD as compared to symptomatic smokers without airway obstruction (26.6±11.5 μg/ml vs 1.9±0.8 μg/ml; $p<0.0001$). Among COPD patients, HNP concentrations were significantly higher in patients with stages 3 and 4 as compared to patients with mild to moderate disease (31.7±8.4 μg/ml; vs 12.3±4.8 μg/ml, $p<0.0001$).
Importantly HNP sputum levels showed a highly significant negative correlation with FEV1 and with FEV1/FVC (r= -0.75, p<0.000001 and r=0.4, p =0.000003 respectively).
Conclusion: Our findings suggest that HNP, a major product released by activated neutrophils, may have a role in monitoring lung inflammation and may be used to trace airway obstruction during COPD clinical course.

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Comparison between Penh and airway resistance in a mild and severe mouse model for allergic asthma

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Background: For many years the parameter Penh to measure airway hyperresponsiveness has been under discussion.
Objective: Compare invasive ventilated lung resistance in anesthetized mice with the non-invasive non-ventilated Penh measurement in conscious mice in a mild and severe allergic asthma model.
Methods: In Balb/c mice airway hyperresponsiveness and inflammation were determined. Intraperitoneal sensitization and aerosol of ovalbumine was used to induce mild asthma. Intraperitoneal sensitization with trinitrophenyl-ovalbumin followed by intranasal challenge with trinitrophenyl-ovalbumin-IgE was used to induce severe asthma. One hour after lung function measurement bronchoalveolar lavage was performed.
Results: A significant increase in airway responsiveness to methacholine was observed in the mild group when lung resistance was measured but not with the Penh. The increased airway responsiveness was much more pronounced in the severe group and significant changes were observed using lung resistance measurement or Penh. In the mild group there was a significant increase in number of inflammatory cells in the bronchoalveolar lavage which was more pronounced in the severe group. Interestingly, ventilation of the animals after the lung resistance measurement, increased even further the number of cells in the bronchoalveolar lavage.
Conclusion: Although the Penh is under discussion, it seems that ventilation of the animals increases the numbers of bronchoalveolar lavage cells independent of the severity of asthma. The acute increase of inflammatory cells due to ventilation, may contribute to the more pronounced increase in airway responsiveness using the lung resistance measurement.