95. Inflammatory mechanisms in COPD

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Procalcitonin as a diagnostic marker in acute exacerbation of COPD

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Background: Rational prescription of antibiotics in acute exacerbations of COPD (AECOPD) requires predictive markers. Recently, measurement of procalcitonin (PCT) levels appears to be useful in order to minimize this problem. We aimed to evaluate the diagnostic and prognostic role of procalcitonin in (AECOPD).
Patients and methods: A total of 50 patients with AECOPD and 10 of apparently healthy individuals (control group) were studied. On presentation, serum PCT concentrations were measured, and quantitative sputum culture was performed for AECOPD. The patients were reevaluated when they had returned to their stable clinical state. The patients were classified into two subgroups: group A included patients with bacterial AECOPD (n = 20), group B included patients with nonbacterial AECOPD (n = 30).
Results: On presentation, the levels of PCT for patients of group A (2.69±0.62 ng/mL) were significantly higher than group B (0.07±0.02 ng/mL) and control group (0.05±0.02 ng/mL) ($P < 0.001$). When they had returned to their stable state, the levels of PCT for patients of group A decreased to (0.06±0.03 ng/mL), which was significantly lower than that in exacerbation (2.69±0.62 ng/mL) ($P < 0.001$); But in patients of group B compared with exacerbation the levels of PCT did not changed (0.068±0.02 ng/mL) ($P>0.05$). Asignificant correlation was recorded between PCT levels in group A and temperature ($r=0.898$, $p<0.05$), leucocytic count ($r=0.889$, $p<0.05$), FEV1% of predicted ($r=0.898$, $p<0.05$),
Conclusions: Procalcitonin is a good marker for differentiation between bacterial and nonbacterial AECOPD and could be used to guide antibiotic therapy and reduce antibiotic overuse in hospitalized patients with AECOPD.

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Transforming growth factor-beta1 (TGF-β1) expression is related to reticular basement membrane (Rbm) hypervascularity in smokers and COPD

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Introduction: TGF-β1 is likely to play an important role in COPD airway pathology, including angiogenesis and epithelial mesenchymal transition (EMT), but it is relatively under-investigated in this condition. We have previously published that the Rbm is fragmented as a likely marker of active EMT and hyper-vascular in the airways of current smokers either with or without COPD.
Objective: This study evaluated the status of TGF-β1 in endobronchial biopsies (ebb) from smokers with or without COPD.
Methods: Ebb sections from 15 smokers with normal lung function (S-NLF), 19 current (CS) and 14 ex-smokers (ES) with COPD were immunostained for TGF-β1 and compared to 17 normal controls (NC). The percentage area of tissue and the number and area of vessels and also the percentage of vessels staining positively for TGF-β1 were compared between groups.
Results: There were no differences between groups in epithelial TGF-β1 staining. TGF-β1 stained vessels in the Rbm were increased in S-NLF, CS-COPD and ES-COPD compared to NC, but especially so in CS-COPD [median (range) for number of vessels/mm Rbm 2.5 (0.0-12.7), 3.4 (0.0-8.1) and 1.0 (0.0-6.3) vs. 0.0 (0.0-7.0), $p<0.05$]. Percentage of vessels stained was also increased in these clinical groups compared to NC [median (range) for S-NLF 31% (0-121), for CS-COPD 40% (0-123) and for ES-COPD 22% (0-114) vs. H-N 0% (0-26), $p<0.05$].
Conclusions: Vessel-associated TGF-β1 was increased in smokers and COPD, but especially in CS-COPD. This is likely to be relative to the pathogenesis of COPD; EMT, structural remodelling, angiogenesis and tumorigenesis.

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Blood outgrowth endothelial cells are senescent and dysfunctional in COPD due to increased DNA damage; implications for endothelial dysfunction

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Introduction: Cardiovascular disease (CVD) is a major cause of death in COPD. The molecular pathways that lead to endothelial dysfunction and CVD in COPD remain unclear. DNA damage has been recognized as an important contributor in aging disorders. Blood outgrowth endothelial cells (BOEC) -alternatively named Late endothelial progenitor cells- could serve as a research tool to investigate endothelial defects in COPD patients.
Aim and objectives: To examine whether BOEC exhibit increased DNA damage linked to dysfunctional characteristics, illustrating the underlying molecular process of endothelial dysfunction in COPD.
Methods: BOEC were isolated from peripheral blood samples received from 16 healthy non-smokers (age ± SEM, 57±2.7yr; 5 males), 10 healthy smokers (57±2.6yr; 5 males) and 16 COPD patients (67±1.6yr; 11 males). DNA damage was assessed by measuring two markers of double-strand break formation, 53BP1 and γ-H2AX, by immunostaining. Endothelial senescence was measured by senescence-associated β-galactosidase (SA-β-Gal) activity, and sirtuin (SIRT)1 protein levels by Western blotting.
Results: BOEC from smokers and COPD patients showed markers of increased DNA damage and displayed significantly reduced SIRT1 protein levels and increased senescence compared to healthy non-smokers.
Conclusions: The results from our study demonstrate that BOEC from smokers and COPD patients display increased DNA damage linked to epigenetic molecular dysfunction and increased senescence. These defects may contribute to endothelial dysfunction and cardiovascular events in smokers and patients with COPD.

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Increased levels of soluble intercellular adhesion molecule 1 in active smokers

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Objectives: Serum intercellular adhesion molecule-1 (sICAM-1) is known to be a smoking-associated inflammatory marker but data on the relationship between active smoking and sICAM-1 are lacking for COPD. In the present study we collected a group of COPD patients and non-COPD smokers and measured the sICAM-1 in order to provide information on its expression related to active smoking.
Methods: This report is based on a cross sectional analysis of a case-control study, in which 141 COPD cases and 56 controls (non-COPD smokers) were consecutively recruited. Clinical information from all subjects was registered using a designed questionnaire that included direct questions on co-morbid conditions, respiratory symptoms and tobacco history. Peripheral blood concentration of sICAM-1, together with interleukin-8 (CXCL8), C-reactive protein (CRP), and serum amyloid A (SAA) were determined in all cases.
Results: There were 89 ex-smokers and 108 active smokers of them in the sample. CRP and SAA (log-scale) were elevated in patients with COPD as compared to control subjects ($p = 0.005$ for CRP and $p = 0.024$ for AAS). SAA and sICAM-1 were associated with active smoking in the bivariate analysis. ICAM-1 retained this association when corrected by age, gender, the presence of COPD, inhaled corticosteroids use, body mass index, and FEV1 as covariates.
Conclusion: The present study confirms an association between sICAM-1 levels and active smoking in a group of COPD and non-COPD smokers. This association is specific of ICAM, not affecting other COPD-related biomarkers.

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Correlation between immunohistochemical CuZn-SOD expression and the histopathological features in small airways of patients with severe COPD

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The central causative factor of COPD is cumulative oxidative stress as result of long-term tobacco smoking and an extensive inflammatory response. Superoxide dismutases (SODs) are the primary superoxide-scavenging enzymes in mammalian tissues. This study investigate the relationship between inflammatory and SODs profiles in different small airways compartments according to the severity of COPD and local destructive index (DI).
The localizations of macrophages (MQ), mast cells, neutrophils, CuZn-SOD, Mn-SOD, and EC-SOD were investigated by immunochemistry using paraffin-embedded sections from 15 controls (nonsmokers+smokers), and 25 subjects with mild-to-very severe COPD. Histological/immunohistochemical analyses were performed using the same Region-of-Interest within each small airway.
Significant ($p<0.05$) up-reglation of CuZn-SOD was observed in bronchiolar epithelium, pulmonary vessels, and alveolar parenchyma in very severe COPD, whereas epithelial Mn-SOD and EC-SOD expressions were less affected. The percentage of CuZn-SOD was higher in alveolar parenchyma in moderate/severe COPD but this did not reach statistical significance. Higher MQ and neutrophils densities were found in the interstitium in very severe COPD. Statistically significant correlation was found between MQ/Mn-SOD and EC-SOD within the small airways and MQ/Mn-SOD in the alveolar parenchyma.
In the small airways of patients with very severe COPD there is up-regulation of CuZn-SOD and correlates with the lower DI. The SODs had a patchy distribution in all compartments, where the intensity varied between micro-localization within small airways and alveolar parenchyma.

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The impact of long-term tobacco smoking on circulating IL-16$^+$ NK cells

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Natural killer (NK) cells constitute a first line of anti-viral host defence and tobacco smoke may cause reduced cytotoxicity. Among the cytokines expressed in NK cells, interleukin-16 (IL-16) is of interest since it is known that the extracellular concentrations of this CD4 cell chemoattractant are increased in the airways of long-term smokers. Here, we investigated whether long-term smoking alters the number and IL-16 content of circulating NK cells.
Never-smokers (NS) and asymptomatic smokers (AS) with a normal ventilatory capacity plus a normal diffusion capacity for carbon monoxide (DLCO) were included. We also examined smokers with COPD (GOLD stages 2 & 3) with reduced DLCO (>2SD from the predicted mean). In each subject, a peripheral, venous blood sample was taken during clinically stable conditions for flow cytometry analysis of intracellular IL-16 in NK cells (IL-16$^+$ NK cells; IL-16$^+$CD3$^-$CD16$^+$CD56$^+$). The relative and absolute number of NK cells (CD3$^-$CD16$^+$CD56$^+$) was determined.
Smokers (AS and COPD) exhibited a lower relative number of IL-16$^+$ NK cells compared to NS (Mann-Whitney U test, $p<0.05$). In line with this, the absolute number of IL-16$^+$ NK cells tended to be lower in smokers as well, although this trend was not statistically significant. Among smokers, there was a negative and statistically significant correlation for both the absolute and relative numbers of NK cells, on the one hand, and tobacco load (ie. pack-years; Spearmen Rank Corr. test; $p<0.05$; rho=-0.056 for both correlations) on the other.
Our study indicates that long-term smoking exerts a negative impact on circulating NK cells, in terms of number and IL-16 content. Hypothetically, this impact may impair anti-viral host-defence.

P787

The dynamics of IV-type collagen contents in BALF of patients with COPD

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Background: Chronic obstructive pulmonary diseases are diagnosed in 4-6% of men and 1-3% of women above 40 years old. The persistent inflammatory process in bronchi, the development of microcirculation disorders, the increasing of hypoxia processes result in the activation of fibroblasts and their production of IV-type collagen, which is manifested by the formation of peribronchial pneumosclerosis.
Purpose of the study: The evaluation of the IV-type collagen level dynamics in BALF of patients with COPD.
Materials and methods: The contents of IV-type collagen in BALF has been evaluated by the Enzyme-linked immunosorbent assay method in 28 patients with 2nd stage of COPD in a relapse phase, and then in the same patients again in 6,4±1,2 years during the transformation of the diagnosis into 3rd stage of COPD.
Results of the study: The contents of IV-type collagen in BALF with II stage COPD in the relapse phase was (38,61±2,12) ng/ml, which is 3,99 times higher than in almost healthy people whose level was (9,68±0,54) ng/ml. The average FEV1 magnitude was 57,4±4,2%. During the progressing of COPD and its transformation into 3rd stage, the average magnitude of the FEV1 indicator was 43,4±3,6%; the IV-type collagen level in BALF rose up to 35% in comparison with previous data and was equal (52.13±3.12) ng/ml.
Conclusions: The progressing of COPD is accompanied by the increase of the remodulation of bronchial tree due to peribronchial pneumosclerosis, which is manifested by the IV-type collagen levels rise.

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Senescence marker protein-30 decreases oxidative stress in human lungs of smokers with chronic obstructive pulmonary disease

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Introduction: Senescence marker protein-30 (SMP30) reportedly protects mice lung from oxidative stress associated with smoking. Little is known about the presence of SMP-30 in lungs of chronic obstructive pulmonary disease (COPD).
Methods: Lung tissue was examined from 47 subjects undergoing resection for peripheral lung tumors as follows: current smokers with (n= 9) and without COPD (n= 7), ex-smokers with (n= 8) and without COPD (n= 8), nonsmokers with (n= 7) and without COPD (n= 6). SMP-30 was investigated by immunohistochemistry in lung tissue specimens and Western analysis, qRT-PCR in total lung homogenates. Morphologic evaluations of the lungs, glutathione, malonaldehyde (MDA), interleukin-8 (IL-8) and tumor necrosis factor-α in the lung tissues were determined.
Results: Weak SMP-30 protein was localized predominantly in the cytoplasm of bronchial epithelial cells. A notable decline of SMP-30 mRNA and protein was found in lung tissue of patients with COPD compared to healthy subjects ($P<$ 0.05), also in smokers and ex-smokers with or without COPD when compared with spirometry matched nonsmokers. Inverse correlation was observed between SMP-30, MDA, IL-8 and alveolar destructive index ($P<$ 0.05).
Conclusions: SMP-30 decreases oxidative stress from smoking and pulmonary inflammation, which may contribute to protecting smokers from susceptibility to the development of COPD.

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Relation of inflammatory process with COPD phenotype

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Aim: Chronic obstructive lung disease (COPD) does not have a uniform clinical and morphological nature. The aim of the study was to characterize the inflammatory process in two distinct forms of COPD (chronic bronchitis or emphysema).
Methods: 33 COPD patients were investigated. They were divided into two groups: emphysema (n=15) and chronic bronchitis patients (n=18). The distinguishing criteria were the presence of emphysema in HRCT and hyperinflation in pulmonary function test.
The concentration of inflammatory mediators in BAL (IL-8, TNFα, myeloperoxidase (MPO), and neutrophil elastase (NE)) and blood (IL-8, TNFα, CRP and fibrinogen) were measured.
Results: The severity of obstruction (FEV1%) was similar in the two groups (p=0.57). The median BAL concentration of inflammatory mediators (IL-8, TNFα, MPO, NE) did not differ between two groups (p values were 0.82, 0.10, 0.69, and 0.49, respectively). Also serum concentrations of IL-8, TNFα, CRP and fibrinogen were similar between chronic bronchitis and emphysema patients (p values were 0.92, 0.50, 0.32, and 0.53, respectively).
Conclusions: We have shown no differences between the two COPD phenotypes in respect of systemic and bronchial inflammatory profile. This result may indicate a possible similar character of inflammation in chronic bronchitis and emphysema phenotypes.

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MMP-9 expression and activity is incresed in the BAL of patients with acute exacerbation of COPD

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Background: Exacerbations of COPD are associated with accelerated deterioration of respiratory symptoms and pulmonary function. Matrix metalloproteinases (MMP) are a family of endopeptidases involved in extracellular matrix cleavage. We hypothesize that there is a difference in the expression and the enzymatic activity of MMP in bronchoalveolar lavage fluid (BALF) of COPD patients with and without exacerbation.
Methods: Seventy patients with COPD diagnosed according to the GOLD guidelines with either stable disease or exacerbation and undergoing diagnostic bronchoscopy were included in this prospective cohort study. BAL was performed by installation of 3 x 50 ml of pyrogen-free sterile NaCL 0.9% solution over the working channel of the bronchoscope according to standard guidelines. Enzymatic activity of MMP was assessed by gelatin zymography and protein levels of MMP-2, MMP-9 and of tissue inhibitors of MMP (TIMP) were measured by ELISA.
Results: Data of 40 patients with COPD (stable COPD n=20, exacerbation n=20) have been analyzed so far. Mean age was 68.9 years (±9.5), mean FEV1%pred 49.9% (±16.9), mean DLCO %pred 43.1% (±18.8). As compared to patients with COPD at stable state, the MMP-9 enzymatic activity and protein expression were increased by 73% and 62%, respectively ($p<0.001$) in the BALF of patients with COPD at exacerbation. In contrast, there was no significant alteration in TIMP expression, indicating a net increase of collagenase activity associated with exacerbation.
Conclusion: Increased activity of MMP-9 during acute exacerbation might contribute to tissue destruction and development of emphysema in patients with recurrent exacerbations of COPD.

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Enhanced IL-6 and CCL3 activity in COPD

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Rationale: IL-6 is a pleiotropic cytokine that is involved in the regulation of inflammation. Increased serum IL-6 levels are associated with reduced FEV1 in COPD patients independent of age or smoking status. Elevated levels of sputum IL-6 in COPD patients have been associated with increased exacerbation frequency. The mechanism by which IL-6 may mediate inflammation in COPD is uncertain. We sought to determine levels of IL-6 and its soluble receptor (sIL-6R) in COPD sputum. IL-6 signaling can alter the levels of the neutrophil chemoattractant CCL3 and the monocyte chemoattractant CCL2; we also investigated the levels of these chemokines.
Methods: 70 patients with GOLD stage I-IV COPD and 30 healthy controls comprising of 15 healthy smokers (HS) and 15 healthy non-smokers (HNS) underwent sputum sampling with PBS processing. Levels of IL-6, sIL-6R, CCL2, CCL3 were determined by multiplex analysis (MSD® platform) of sputum supernatant.
Results: Healthy smokers expressed the highest levels of sputum IL-6. COPD patients expressed the highest levels of sIL-6R. COPD patients also expressed the highest levels of sputum CCL3. In contrast, CCL2 expression was significantly reduced in COPD patients.

	COPD	HS	HNS	ANOVA
IL-6 (pg/ml)	116.8 (84.5)	258.3 (214.1)	80.87 (74.34)	p=0.0016
sIL-6R (pg/ml)	256.6 (260.9)	98.84 (44.33)	136.2 (90.31)	p=0.019
CCL2 (pg/ml)	26.7 (51.2)	207.8 (104)	162.4 (142.6)	p<0.0001
CCL3 (pg/ml)	182.1 (164)	8.12 (6.83)	58.75 (63.17)	p<0.0001

Data expressed as mean (SD).

Conclusion: We report evidence of enhanced IL-6 signaling and CCL3 activity in COPD sputum. We have observed that there is reduced CCL2 activity and enhanced CCL3 activity in COPD sputum. IL-6 may therefore promote neutrophillic inflammation in COPD through up-regulation of CCL3 expression.

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Differences in cellular expression of C-reactive protein and serum amyloid A in lung tissue in patients with chronic obstructive pulmonary disease

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Introduction and objectives: The lung bronchial and parenchyma tissues are a potential source of acute phase reactants in Chronic Obstructive Pulmonary Disease (COPD) patients as compared with resistant smokers. The aim of this study was to determinate the expression of C-Reactive Protein (CRP) and Serum Amyloid A (SAA) in epithelial cells, macrophages and lung fibroblasts between COPD and resistant smokers. This expression was also studied according to the different grades of COPD.
Method: This report is based on a cross sectional analysis of a case-control study. These patients included were consecutively recruited, in elective lung surgery.Epithelial cells, macrophages and fibroblasts were obtained by magnetic separation microbeadsand CRP and SAA1, 2 and 4 expression was analysed by real time PCR.
Results: The sample was formed by 19 COPD and 27 resistant smokers. Although all cell types were able to synthesize the biomarkers, fibroblasts of COPD patients had a significantly higher expression (5 folds, p=0,015) of SAA1 than resistant smokers. Our results also showed significant differences in the expression of SAA between macrophages from COPD in different stage of the disease, being higher in patients in GOLD II (25 folds higher for SAA1, p=0,021; 9 folds for SAA2, p=0,05 and 30 for SAA4, p=0,014).
Conclusions: There are differences in the synthesis of SAA-1 in fibroblasts of COPD and controls. The pattern of expression in macrophages is different for SAA according to stage of disease.These findings could be useful to elucidate the contribution of each cellular compartment in the inflammatory component of the disease.

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Analysis of reactive oxygen species in sputum neutrophils during acute exacerbation of COPD

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Background: Chronic airway inflammation in COPD can be mediated by enhanced oxidative burst in neutrophils. Our previous study showed enhanced production of reactive oxygen species (ROS) in peripheral blood neutrophils (PBN) in stable COPD (SCOPD) and during acute exacerbation of COPD (AECOPD).
Aim of the study: To analyze ROS production in sputum neutrophils during AECOPD.
Methods: Thirty-five patients during AECOPD and the same patients in SCOPD, as well as 10 healthy individuals (HI) were involved in to the study. Neutrophils were isolated by high density gradient centrifugation and stimulated with PMA (0.1-30 nM) and S. aureus (1-167 bacteria/neutrophil). ROS production was analysed by flow cytometer.
Results: Spontaneous ROS production in sputum neutrophils was 45.8±3.8% and in PBN 29.7±4.1% higher during AECOPD than in SCOPD ($p<0.05$). The most significant increase of ROS production was documented after neutrophil stimulation with 30 nM of PMA (in sputum neutrophils – during AECOPD 450±28-fold, in SCOPD 188±19.2-fold, HI 80±11.5-fold, respectively, ($p=0.01$); in PBN - during AECOPD 246±19-fold, in SCOPD 162±24.2-fold, HI 118±18-fold, respectively, ($p<0.05$)).The intensive ROS production in neutrophils after stimulation with S. aureus was found in AECOPD group compared with SCOPD and HI ($p=0.01$).
Conclusions: ROS production in sputum and peripheral blood neutrophils after stimulation with PMA and S. aureus was more intensive during AECOPD compared with SCOPD. Sputum neutrophils produce higher levels of ROS compare with PBN.

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Elevated sputum complement factor H levels in COPD: Relationship with disease severity

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Background: Inflammatory processes in COPD are not fully clarified yet. Complement activation products C3a and C5a are elevated in COPD sputa, but complement regulatory proteins, such as factor H, have not been investigated in this disorder.
Objective: Our primary goal was to determine airway complement factor H levels in stable and exacerbated COPD in order to gain information about the relationship between factor H level, complement activation and clinical characteristics of disease groups.
Methods: We examined complement factor H levels and SC5b-9, a marker of complement activation in plasma and sputa of 15 healthy, 15 stable and 17 exacerbated COPD subjects by ELISA.
Results: Factor H and SC5b-9 levels were both higher in sputa of stable COPD patients compared to healthy controls (Factor H: $p<0.01$, SC5b-9: $p=0.03$), which further increased in acute exacerbation (Factor H: $p<0.0001$, SC5b-9: $p=0.02$), and returned to stable level after 5-7 days of systemic corticosteroid treatment. Plasma concentrations showed similar tendencies, but with much smaller differences. There was a significant positive relationship between sputum factor H levels and FVC (%, $r=0.71$, $p<0.01$) as well as FEV1 (%, $r=0.57$, $p=0.04$) values in stable patients. Sputum SC5b-9 concentrations correlated with FEF25-75 (%, $r=0.55$, $p=0.04$) in stable COPD.
Conclusions: Increased sputum factor H level is associated with milder airway obstruction in stable COPD, which may be connected to early inflammatory processes preceeding extensive alveolar destruction.
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Stage-dependent regulation of brain-derived neurotrophic factor and transforming growth factor-β1 in patients with COPD

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Chronic Obstructive Pulmonary Disease (COPD) is characterised by complex inflammatory, neuronal and fibrotic changes. Brain-derived Neurotrophic Factor (BDNF) and Transforming Growth Factor-β1 (TGF-β1) are stored in alpha-granules of platelets. Serum BDNF and TGF-β1 are predominantly platelet-derived (released from platelets during serum preparation). BDNF is a key regulator of neuronal plasticity, whereas TGF-β1 is involved in tissue repair and emphysema pathogenesis. We have previously shown that serum BDNF but not TGF-β1 is elevated in asthma, correlating with disease severity. The present study aimed to investigate serum concentrations of BDNF and TGF-β1 in different stages of COPD compared to non-COPD controls. 63 patients with stable COPD (GOLD 2: n=22, GOLD 3: n=28, GOLD 4: n=13) and 17 age- and comorbidity-matched controls without COPD were enrolled. Serum levels of BDNF and TGF-β1 were measured using ELISA. Serum levels of BDNF and TGF-β1 were significantly elevated in all stages of COPD as compared to controls. Highest BDNF concentrations were found in GOLD stage 3 (with a trend towards a decrease in GOLD stage 4), whereas highest TGF-β1 serum levels were found in GOLD stage 4. In contrast to asthma, COPD appears to be characterised by increased concentrations of both BDNF and TGF-β1. Very severe COPD is associated with highest TGF-β1 concentrations, but relatively lower BDNF concentrations. We thus speculate that these findings might reflect a maximum of neuronal and inflammatory activity in GOLD stages 2 and 3, and a predominant activity of tissue remodeling factors in GOLD stage 4.

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IL-4, IFN-gamma and TNF-alpha levels in serum of patients with COPD, bronchial asthma and GERD

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Gastroesophageal reflux disease (GERD) is tightly linked to bronchial asthma and COPD. In our study we evaluated serum of 54 patients. Diagnoses was as follows: asthma (n=14), COPD (n=12), GERD (n=20), asthma and GERD (n=8). Serum of 19 volunteers was studied as a control group. IL-4, IFN-gamma and TNF-alpha levels were detected by ELISA. All patients underwent upper gastrointestinal endoscopy and spirometry. For statistics nonparametric method of Kruskal-Wallis and Spearman's correlation were used.
Results: The IL-4 and IFN-gamma levels in all groups of patients were significantly higher than control ($p=2.5E-09$ and $1.76E-09$ respectively). Meanwhile TNF-alpha values in patients cohorts was lower than in control group ($p=0.007$). Patients were divided into three groups according to endoscopy: (i) no symptoms (n=6), (ii) chronic gastritis (n=27), and (iii) reflux esophagitis (n=21). The level of IFN-gamma was lowest in no symptoms group (91.6±41.9 pg/mL), intermediate in gastritis patients (204.2±255.8 pg/mL), and highest in reflux eosophagitis group (404.8±455.7 pg/mL) – $p=0.01$. IFN-gamma/IL-4 ratio had similar dynamics ($p=0.01$). TNF-alpha value was maximal in reflux esophagitis group ($p=0.0004$). We investigated cytokines values depending on severity of respiratory failure. In patients with no respiratory failure level of IL-4 was minimal, stage 1 patients had medium elevation of IL-4 value, and in patients with stage 2 of RF level of cytokine was maximal ($p=0.001$).
Conclusion: In our study IL-4 exhibited a significant role in the severity of respiratory disorders, whereas IFN-gamma and TNF-alpha were determined degree of damage to the gastrointestinal tract.

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C-reactive protein and serum amyloid A overexpression in lung tissues of chronic obstructive pulmonary disease patients

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Background: Although researchers have consistently demonstrated systemic inflammation in chronic obstructive pulmonary disease (COPD), its origin is yet unknown. We aimed to compare the lung bronchial and parenchymal tissues as potential sources of major acute-phase reactants in COPD patients and resistant smokers.
Material and methods: Consecutive patients undergoing elective pneumectomy or lobectomy for suspected primary lung cancer were considered for the study. Patients were categorized as COPD or resistant smokers according to their spirometric results. Lung parenchyma and bronchus sections were obtained and C-reactive protein (CRP) and serum amyloid A (SAA1, SAA2 and SAA4) were studied by RT-PCR and immunohistochemistry.
Results: Our study included 85 patients with COPD and 87 resistant smokers. In bronchial and parenchymal tissues, both CRP and SAA genes were overexpressed in COPD patients as compared to resistant smokers. In the bronchus, CRP, SAA1, SAA2, and SA4 expressions in COPD patients were 1.89-fold, 4.36-fold, 3.65-fold, and 3.9-fold the control values, respectively. In the parenchyma, CRP, SAA1, and SAA2 gene expressions were 2.41-, 1.97-, and 1.76-fold the control values, respectively. SAA4 was not overexpressed in the parenchyma. The expressions were higher in the parenchymal tissue than in bronchial tissue for both COPD and controls. The protein analysis supported the results obtained in the PCR.
Conclusions: These results indicate an overexpression of CRP and SAA genes in both bronchial and parenchymal tissue in COPD. This expression differs between the parenchyma and bronchial tissue, indicating tissue/cell type specificity of these markers.

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T cell chemokine receptor expression in COPD

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Background: COPD is characterized by accumulation of T cells in the lung.

Recruitment is regulated by chemokines binding to receptors on the cell surface. We studied the expression of chemokine receptors on T cells from neversmokers, smokers with normal lung function and COPD patients.
Methods: Thirtyseven neversmokers, 38 smokers with normal lung function and 32 COPD patients, GOLD stage I-II (23 smokers and 9 exsmokers) underwent BAL (5x50 mL). BAL and blood T cells were analysed for CD3, CD4 and CD8 in combination with the activity marker CD69 and the chemokine receptors CXCR3, CCR4 and CCR5 using multicolor flow cytometry.
Results: The percentage of CD4+CD69- (non-activated) T cells expressing CXCR3 was significantly lower in BAL from "normal" smokers and from COPD smokers compared to neversmokers ($p<0.001$ and $p<0.05$). CD4+ T cells from "normal" smokers had significantly higher median fluorescence intensity (MFI) of CCR5 compared to neversmokers ($p<0.05$). An increase, albeit not significant, was also observed in COPD patients who were current smokers. The expression of CXCR3, CCR4 and CCR5 on CD8+ T cells in BAL did not differ. In blood from COPD patients (both current and ex-smokers), we observed a higher percentage of activated (CD69+) CD8+ T cells expressing CXCR3 compared to "normal" smokers ($p<0.05$ for both).
Conclusions: The lower percentage of CD4+CD69-CXCR3+ T cells and the higher MFI of CCR5 on CD4+ T cells in BAL from both smoking groups seem to be related to smoke exposure *per se*, rather than the degree of airway obstruction. This was not observed in COPD exsmokers, indicating that both smoking history and current smoke exposure affect the expression. Analysis of soluble ligands for CXCR3, CCR4 and CCR5 is in progress.

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Roflumilast-N-oxide exerts anti-remodelling potencies in COPD patients *in vitro*
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COPD is characterised by progressive airway remodelling and peribronchiolar fibrosis. The origin of pathologic remodelling in COPD is unknown and may result from local hypoxia and increased growth factor expression, including transforming growth factor-beta (TGF-β). TGF-β increases the synthesis of collagen1A1, connective tissue growth factor (CTGF), alpha smooth muscle actin (SMA). This study explores whether the phosphodiesterase-4 (PDE4) inhibitor roflumilast N-oxide (RNO), the active metabolite of roflumilast in use for severe COPD modifies TGF-β induced ECM composition.
Methods: Lung fibroblasts from patients with COPD (GOLD II-III) (n=3) were isolated and cultured using standard protocols. Cells were preincubated with RNO (1μM), Budesonide (BUD, 100nM), or vehicle (0.1% DMSO) and stimulated with TGF-β (1 or 2ng/ml) for 24 hours. CTGF, Collagen (Col) 1A1, and SMA mRNA expression were measured by quantitative RT-PCR (18S mRNA served as reference gene) and results were depicted as relative expression.
Results: In COPD lung fibroblasts, TGF-β induced CTGF (1.2 fold (SD±0.19) p=0.0069), Col1A1 (1.51/±0.41/p=0.003) and SMA (1.6/±0.15/p=0.0028) significantly, key modulators of extracellular matrix composition *in vitro*. The addition of RNO significantly reversed this induction after 24 hours for all parameters (CTGF:-21%, Col1A1:-23%, α-SMA:-30%), whereas BUD did not exert such an inhibitory effect for SMA and CTGF.
Conclusion: Roflumilast N-oxide diminished TGF-β-induced gene transcription of different markers of remodelling (Col1A1, SMA, CTGF) in isolated human primary fibroblasts of COPD patients. These findings may support the notion of roflumilast N-oxide mitigating a fibrotic response in COPD.