329. Role of infection in exacerbations of COPD

P3008

Human metapneumovirus involved in acute exacerbations in COPD patients?

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Introduction: COPD is characterized by the occurrence of acute exacerbations (AE) which may be caused by several factors, including infections. Human metapneumovirus (hMPV) is an important cause of severe respiratory tract infections in young children, the elderly and immunocompromised subjects. This study evaluates the presence of hMPV and other respiratory viruses in stable sputum samples of patients with COPD and the relation with the occurrence of AE.
Material and methods: From March 2009 until August 2010 sputum samples of COPD patients clinically rehabilitating at the centre of expertise for chronic organ failure (CIRO), were included. Patients were followed during an 8 week period and sputum samples were collected during the stable state and during an AE. Real-time PCR for respiratory viruses, including rhinovirus and hMPV, was performed.
Results: A total of 498 sputum samples (218 patients) were included, 257 samples (52%) were collected during the stable phase. Viruses were detected in 64 (25%) of the stable samples. The most frequently isolated viruses in the stable samples were hMPV (12%) and rhinovirus (12%). A total of 74 patients experienced an AE in the weeks after stable sample collection, of which 12 (16%) had hMPV in the stable sample. Patients with hMPV in the stable phase experienced significantly more AEs in the first weeks after sample collection compared to patients without hMPV (or any other virus) (p=0.03).
Conclusion: hMPV was one of the most isolated viruses in samples collected during the stable state. Patients with hMPV in the stable sample experienced more exacerbations compared to patients without any virus in the stable sample.

P3009

Amoxicillin concentrations in sputum in relation to betalactamase activity in COPD patients

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Introduction: COPD exacerbations are often treated with antibiotics, although their use is controversial. A previous study showed that patients with a sputum concentration of amoxicillin (a beta-lactam antibiotic) lower than the minimum inhibitory concentration (MIC90, value 2 mg/l) were hospitalized 4 days longer then patients with a concentration ≥MIC90. One explanation for a low amoxicillin concentration could be that patients' lungs are colonized or infected with pathogens that have beta-lactamase activity.
Objective: This study investigated if beta-lactamase activity was higher in patients who had a sputum amoxicillin concentration <2 mg/l than in patients with a concentration ≥2 mg/l.
Methods: 23 Hospitalized COPD patients treated with amoxicillin/clavulanic acid for an acute exacerbation were included. Sputum and serum samples were collected at the third day of treatment to determine beta-lactamase activity in sputum and amoxicillin concentrations in both sputum and serum.
Results: We found no difference in beta-lactamase activity between patients with a sputum amoxicillin concentration <MIC90 and ≥MIC90 (p=0.79). Amoxicillin concentrations were <MIC90 in 18 out of 23 sputum samples (78%). Serum concentrations amoxicillin were <MIC90 in 7 patients (30%).
Conclusions: Beta-lactamase activity did not differ between patients with sputum amoxicillin concentrations <MIC90 or ≥MIC90. The finding that a majority of patients had a sputum amoxicillin concentration <MIC90 is troubling. This suggests that these patients are being undertreated. Further research could focus on inhalation of amoxicillin to obtain higher concentrations in sputum.

P3010

Clinical and economical impact of exacerbations of chronic obstructive pulmonary disease (COPD): A two-year study

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COPD is a common disease with considerable health and economic consequences. The aim of our study was to analyze clinical and economical impact of COPD exacerbations (E-COPD). A secondary data analysis of healthcare administrative databases of Lombardy, Italy was performed, including patients hospitalized for a severe E-COPD (index event) from Jan to Dec 2003. Two study groups were identified based on the number and type of E-COPD experienced after the index event during a two-year follow-up: those who had at least one severe E-COPD (Group A) and those who had only moderate E-COPD (Group B). A total of 12,436 patients (7,933 males; mean±SD age: 74±10 yrs) were enrolled. Among those, 45% belonged to Group A and 55% to Group B. Clinical outcomes, costs and consumptions of pulmonary function test (PFT) per person during the follow-up are given in Table.

	Group A	Group B
Deaths, n (%) pts*	1,503 (27)	1,502(22)
E-COPD, mean (SD)*	6.3 (4.98)	3.9 (3.85)
Total healthcare costs, mean (95% CI)	9,699 (9,399–9,929)	4,647 (4,526–4,779)
Hospitalization cost, mean (95% CI)	7,688 (7,425–7,918)	2,916 (2,813–3,026)
Prescription cost, mean (95% CI)	1,542 (1,512–1,582)	1,195 (1,168–1,229)
Patients with PFTs, n (%)	1,350 (24)	1,545 (22)
PFTs cost, mean (95% CI)	13 (12–14)	12 (12–13)

Cost in EURO. *p<0.01 Age-adjusted difference between groups.

Mortality risk increased in the study population in relation to the number and type of E-COPD experienced during the follow-up. The number and type of

E-COPD significantly impact outcomes and costs. Less than one fourth of patients discharged after an E-COPD will undergo a PFT during two years, although its cost is extremely lower than the hospital stay.

P3011

Safety and pharmacokinetics of multiple-dose ciprofloxacin dry powder for inhalation in patients with moderate or severe COPD

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Introduction: Many patients with COPD are chronically colonized with bacteria and could benefit from long-term antibacterial therapy. Ciprofloxacin dry powder for inhalation (DPI) is an investigational PulmoSphere™ formulation for pulmonary delivery of ciprofloxacin.
Aim: To investigate the safety, tolerability and PK of ciprofloxacin DPI in patients with COPD.
Methods: In a randomized, phase I, single-blind, parallel-group study, adults with GOLD stage II or III COPD received 32.5 mg or 48.75 mg ciprofloxacin (50 mg, 75 mg ciprofloxacin DPI, respectively) or matching placebo as a single dose on Day 0 and 12 and bid on Days 2–11.
Results: Patients received 32.5 mg (n=6) or 48.75 mg (n=9 including 3 who replaced drop-outs) ciprofloxacin, or placebo (n=4). There were no severe or serious AEs; most AEs were mild in severity. There were treatment-related AEs in 4, 8 and 1 patients in the 32.5 mg and 48.75 mg ciprofloxacin and placebo groups, respectively. Three patients discontinued (48.75 mg ciprofloxacin) due to AEs: moderate treatment-related dyspnoea (n=1); infectious diseases (n=2). Day 0 geometric mean plasma ciprofloxacin AUC was 0.532 mg*h/l and 0.727 mg*h/l, and AUC_{0-last} in induced sputum was 1190 mg*h/l and 2010 mg*h/l, for the 32.5 mg and 48.75 mg groups, respectively. Ciprofloxacin sputum concentrations were highly variable; geometric mean induced sputum C_{max}:plasma C_{max} ratios ranged 1650–10,600.
Conclusions: Ciprofloxacin DPI was well tolerated over 12 days' treatment in patients with moderate or severe COPD. High sputum concentrations contrasted with low systemic exposure.

P3012

Does presence of multiple viruses in the stable state predispose COPD patients to exacerbations?

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Introduction: Respiratory viruses are associated with acute exacerbations (AE) in patients with COPD, but may also lead to asymptomatic carriership. This study investigates whether co-detection of viruses in the stable state predisposes for AEs in patients with COPD. Since it is known that many viruses circulate during the year we included the most important respiratory viruses in our study.
Material and methods: From March 2009 until August 2010 sputum samples of COPD patients clinically rehabilitating at the centre of expertise for chronic organ failure (CIRO), were included. Sputum samples were collected during the stable state and during an AE. Real-time polymerase chain reaction for rhinovirus, hMPV, RSV, influenza A and B and parainfluenza 1-4, was performed.
Results: A total of 498 sputum samples (218 patients) were included. Viruses were detected in 208 samples (42%) of which 106 (51%) were collected during the stable state. In 11% of the samples multiple viruses were found, of which the combination of rhinovirus and hMPV was the most frequent (58%). Patients with multiple viruses in the stable sample did not experience more AEs in the first weeks after stable sample collection than patients with a single virus or without a virus in the stable sample.
Conclusion: In 11% of sputum samples of COPD patients multiple respiratory viruses were found. The presence of multiple viruses does not predispose these patients to the short-term occurrence of an AE.

P3013

The relationship between PBMC secretion of IFN-alpha following TLR7 and TLR9 activation and lung function in COPD

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Introduction: Viral and bacterial infections play a key role in exacerbations in COPD and have been implicated in the persistence of symptoms. The relationship of the inflammatory response to these airway pathogens at stable state and exacerbations is also poorly understood and how this may lead on to structural airway remodelling is poorly defined. We sought to investigate the relationship between clinical parameters in COPD and the *in vitro* response to TLR 7 & 9 activation.
Method:PBMCs were stimulated for 24h, with 10μM R848 and 1μM CpG, from healthy controls & COPD subjects. IFN-α release was measured in supernatant using ELISA kits.
Results:37 COPD & 8 healthy control subjects were recruited. The mean (SD) FEV_1 (L) & FEV_1/FVC of the COPD subjects was 1.32 (0.58) & 0.5 (0.13) respectively. There was no significant difference in IFN-α (pg/ml) release when comparing health 74 (30) to COPD 59 (42) (p=0.33) when stimulating TLR 7. TLR 9 stimulation gave a significant increase in IFN-α production in COPD 204 (106) vs health 114 (61) (p=0.003). TLR9 stimulation was significantly increased compared to TLR7 in COPD (p<0.0001). There was a significant negative correlation with lung function and TLR 7 stimulation in the COPD group, FEV_1 (r=-0.46, p=0.005); FEV_1% predicted (r=-0.37, p=0.024); FEV_1/FVC (r=-0.39, p=0.002). TLR 9 stimulation had a significant negative correlation with gas exchange, KCO% predicted (r=-0.55, p<0.001) and TLCO% predicted (r=-0.43, p=0.01).
Conclusion: The release of IFN-α by PBMCs was increased in COPD compared to healthy controls following TLR 9, but not TLR 7 stimulation. The IFN-α release after TLR7 and 9 activation was related to lung function.

P3014

Bacterial load in chronic obstructive pulmonary disease and its relationship with airway inflammation and lung function

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Introduction: Chronic Obstructive Pulmonary Disease (COPD) is characterised by fixed airflow obstruction, typically in association with persistent airway inflammation and bacterial colonisation. The relationship between these features of disease is not fully understood.
Method: Patients with COPD were recruited from a single centre and were characterised in terms of their lung function, health status, sputum cell counts and quantitative microbiology to derive colony forming units (CFU)/ml of sputum
Results: 126 patients were recruited, 83 were male. The mean (SEM) was for FEV_1% predicted 48.10 (1.64), sputum neutrophil count 73.2 (2.06)%, total cell count (TCC) 6.55 (0.90) 10^6/g, CFU $8.5\times10^7(1.5\times10^7)$ and median (IQR) eosinophil count, 0.75 (0.25-2.5)% data for the neutrophil (%), eosinophil (%).
Univariate correlations was observed between CFU and neutrophil (%) (r=0.306; p=<0.001), TCC (r=0.273; p=0.002), but not eosinophil (%) (r=-0.058; p=0.517) and FEV_1% predicted (r=-0.061; p=0.497).
CFU data was put into tertiles, with a relationship observed with neutrophil (%) (p=0.035), TCC (p=0.028) 10^6/g and eosinophil (%) (p=0.011), but not FEV_1% predicted (p=0.942).
Conclusion: Bacterial load was associated with the sputum total cell count and neutrophil differential but was not associated with lung function. Longitudinal studies are required to assess the role of bacteria in disease progression.

P3015

Induction of cathelicidin (LL-37) in rhinovirus-induced COPD exacerbations

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Cathelicidin (LL-37) is a cationic antimicrobial peptide and has both antimicrobial and immunomodulatory effects. The majority of LL-37 is stored in neutrophil granules for release at sites of infection. LL-37 has demonstrated anti-viral effects but the role of LL-37 in virus-induced COPD exacerbations is unknown.
Methods: We infected 3 groups of subjects–COPD GOLD stage II (N=20), smokers with normal lung function (SMK, N=21) and non-smokers (NS, N=11)–with rhinovirus 16 (RV). Induced sputum was collected post-inoculation. In a subset of COPD (N=10) and SMK (N=12) BAL was collected on day 7. Sputum and BAL cytospins were prepared and cell counts determined. LL-37 and cytokines were measured in sputum supernatants by ELISA and virus load by quantitative PCR.
Results: In all subjects combined post-infection peak sputum LL-37 levels correlated with peak sputum virus load (P=0.0018,r=0.43) and peak sputum total inflammatory cells (P<0.0001, r=0.72). These correlations were also significant in the COPD group alone (P=0.017, r=0.53 and P<0.0001, r=0.85 respectively) but not in the NS or SMK. In BAL LL-37 correlated with BAL virus load (P=0.044, r=0.44), total inflammatory cells (P<0.0001, r=0.89), neutrophils (P<0.001, r=0.89), IL-8 (P<0.0001, r=0.83), TNF-α (P<0.0001, r=0.87) and IL-6 (P=0.002, r=0.63). These correlations were also significant in the COPD group alone but not in the SMK.
Conclusions: LL-37 in sputum and BAL following rhinovirus infection correlates with inflammatory cells, pro-inflammatory cytokines and virus load. Further studies are required to determine whether LL-37 contributes to neutrophilic inflammation in virus-induced exacerbations or is a component of the anti-viral innate immune response.

P3016

Exacerbation frequency is related to PEF self-similarity in COPD

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Introduction: Self-similarity in Peak Expiratory Flow (PEF) can predict the frequency of asthmatic events but it is unknown whether in COPD there is a relationship with exacerbation frequency.
Methods: We examined data from the London COPD cohort on 308 COPD patients who recorded worsening of respiratory symptoms and PEF on daily diary cards. Exacerbations were identified as previously described (Seemungal. AJRCCM 157:1418-1422; 1998) from which an annual rate was calculated. Detrended fluctuation analysis was used to calculate α which is a measure of decay in self-correlation over time (Frey et al Nature. 438: 667-70, 2005). PEF time series when the patient was clinically stable were also analyzed by excluding data collected 7 day before to 7 day after each exacerbation, and splicing the series together.
Results: The 308 COPD patients (195M; mean age (SD) 68.3 (8.4) years; FEV_1 1.12 (0.46) l, FEV_1% predicted 44.5 (16.4), PEF% predicted 54.7 (18.6). The cohort recorded diary card data for a median 1077 days and experienced 2621 exacerbations with a median frequency of 2.04 per year (IQR 0.9 – 3.3). The patients had an α of 0.944 (SD 0.19) over the initial 365 days which was positively related to exacerbation frequency (negative binomial regression, P=0.009). No difference was seen if exacerbation PEF data were excluded, α = 0.935 (SD 0.21; P=0.139) and there was also a relationship with exacerbation frequency (P=0.001).
Conclusion: In COPD patients, long-term correlations (self-similarity) exist in PEF, which are related to exacerbation frequency. The exponent α may prove a useful marker of exacerbation frequency and as normally distributed lead to a reduction in the number of patients required for clinical trials.

P3017

The role of respiratory virus infection in COPD exacerbations

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Aim: To determine the prevalence of viral infection in a cohort of severe COPD patients.
Methods: We conducted a prospective observational study in a cohort of severe COPD patients from 2005 to 2007. We recorded for each exacerbation: etiological agent, prior antibiotic prescription, corticosteroid treatment and days of hospitalization. Sputum samples were collected and the Gram staining Murray-Washington's grade was done. A multiplex retrotranscriptase-nested PCR assay was used for simultaneous detection of Parainfluenza virus (1, 2, 3, 4AB), human Coronavirus 229E and OC43, Enterovirus, Rhinovirus, Influenza virus A, B, C, Respiratory syncytial virus and Adenovirus following the procedure described by Coiras *et al*. (J Med Virol 72:484-95).
Results: A total of 118 patients were registered with a mean age of 69±8 years. The 60% were in GOLD stage IV. Among the 307 respiratory samples processed 89.6% (275) were Murray grade IV-V and 66.1% (203) of them had potentially respiratory bacterial pathogen (positive cultures). Overall, virus were detected in 110/307 (35.8%) of analyzed samples, being Rhinovirus the most common of them (22%). In 51.5% (37/72) of bacterial negative culture samples virus were detected, while in 31% (63/203) of bacterial positive cultures virus co-infection was observed ($p<0.05$).
Conclusions:
– The prevalence of virus infection in our cohort was 36%, being Rhinovirus the most common virus.
– A respiratory viral infection may be suspected in COPD exacerbations among patients with negative bacterial culture.

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P3018

Presence of *Pneumocystis jiroveci* colonization in patients with COPD

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Introduction: *Pneumocystis jiroveci* infections are frequently detected in immunocompromised patients. However, evidence suggests that *P. jiroveci* can be detected in non-immunocompromised patients, where it colonizes the airways. Over the past years, *P. jiroveci* has been linked with COPD. *P. jiroveci* colonization may be associated with the severity of COPD and some evidence suggests a role for *P. jiroveci* in the progression of COPD in smokers. The present study investigates the presence of *P. jiroveci* in sputum samples of patients with COPD.
Material and methods: From March 2009 until September 2010, sputum samples from COPD patients clinically rehabilitating at the centre of expertise for chronic organ failure (CIRO) were included. Patients were followed during an 8 week period and sputum samples were collected during the stable state and during an acute exacerbation (AE). Sputum samples were analyzed for the presence of *P. jiroveci* by a real-time PCR assay.
Results: During the study period 509 sputum samples (218 patients) were collected clinically rehabilitating at CIRO Horn. A total of 184 samples (36%) were collected during the stable phase and 325 (63.8%) during an AE. *P. jiroveci* DNA was detected in 40 (8%) of all sputum samples. A total of 23 positive samples (57.5%) were detected during an AE and 17 during the stable state (42.5%).
Conclusion:
In our population, *P. jiroveci* colonization could be detected in 8% of the sputum samples of COPD patients. Additional research is needed to clarify the role of *P. jiroveci* in the pathophysioloy of COPD.

P3019

Microbiological efficacy of combined use of vaccines against S. pneumoniae, H. influenzae type B in patients with a COPD

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Purpose: To determine the influence of the compatible vaccination "Pneumo-23", "Hiberix" on microbial flora of sputum in patients 12 months after the vaccination.
Materials and methods: The microbiological sputum examination of 31 patients vaccinated with "Pneumo-23", "Hiberix" was made before and 12 months after the vaccination. Pneumococcal bacteria were identified by the sensitivity to optohin, lysis in the presence of bile salts. To isolate H. influenzae paper disks with bacitracin were used, M. catarrhalis was identified by using commercial sets (Gonochek–II), Staphylococcus spp. was revealed by the microscopic method.
Results: In 26 of the 31 patients M. catarrhalis was isolated from the sputum before the vaccination. S. pneumoniae was found in 80,65±7,1% of the cases. In 3 patients K. pneumoniae was isolated, H. influenzae – in 12,9±6,02% of the patients. In 3 patients A. baumanii was isolated, S. spp. – in 6,45±4,41% of the patients, M. odoratus was found in 1 patient.
12 months after the vaccination in 3 patients (9,68±5,31% of the cases, a P value < 0,001) S. pneumoniae was isolated. The statistical significant differences in the number of the patients with H. influenzae, M. catarrhalis, S. spp., K. pneumoniae, M. odoratus, A. baumanii sputum were not determined 12 months after.
Conclusion: The compatible vaccination significantly reduces the occurrence of S. pneumoniae in the sputum of patients with COPD, prevents sputum from the colonization by H. influenzae and S. pneumoniae, may be recommended for use as a prophylactic and therapeutic purposes.

P3020

Etiology pathogens structure in community-acquired pneumonias and COPD exacerbations

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Significance of the microbiology lab diagnostics results in community-acquired pneumoniae (CAP) and exacerbations of COPD still requires evaluation. As the most of them are ubiquitous, the microbiology peculiarities between them remains the keen medical and biological problem. Aim of our research was to define etiology structure of the main microbiology pathogens in CAP patients (1st group) and in COPD exacerbations (2nd group), and to estimate the microbiology differences in isolated strains of S. pneumoniae and H. influenzae.
Materials and methods: We examined 200 adults with CAP and 200 adults with COPD exacerbations of the same age (20-45 years), isolated strains of S.pneumoniae and H.influenzae were checked for antimicrobial agents resistance according to NCCLS standards, typed and studied by MLST.
Results: In the 1st group S. pneumoniae was isolated in 42%, H. influenzae in 26%. In the 2nd group pneumococci were isolated in 36%. Antimicrobial resistance (1st group vs 2nd group) of S. pneumoniae were characterized with resistance to erythromycine (14%/18%), levofloxacine (9%/7%).H. influenzae strains were resistant to co-trimoxazole (20%/27%) and ampicillin (24%/28%). Serotyping of S. pneumoniae revealed that the most of the 2nd group strains were of the serotypes including to the 23-polysaccharide vaccine. H. influenzae strains of the I, II, and III were the prevalent biotypes. MLST of S. pneumoniae showed the prevalence of several lineage clones in CAP strains (1st group).
Conclusion: The microbiological monitoring of the main respiratory pathogens could be significant instrument both in clinical diagnostics and in epidemiology surveillance of the lower respiratory tract infections.

P3021

Characteristic of cytokines levels of blood and sputum in COPD exacerbation

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To study IL-8 and TNF-α levels in blood and sputum in COPD exacerbation in dependence on availability and character of infectional factor.
52 COPD exacerbation patients were examined, infectional nature of exacerbations was proved at 35 patients (first group), infectional factor was not found at 17 patients (second group). The quantitative bacteriological sputum research, definition diagnostic main IgG, IgM levels to Ch.pneumoniae, M.pneumoniae in serum by means of immuno-assay method, in sputum by means of PCR method were made. IL-8, TNF-α levels in sputum and serum were defined by means of hardphase immuno-assay method.
Cytokines levels in the sputum at COPD exacerbation in first group were high than in second group: TNF-α level was 143.1±71.9 pg/ml and 31.0±12.6 pg/ml, (p=0.018), IL-8 level was 1244.5±197.4 pg/ml and 1088.8±222.5 pg/ml, (p=0.021).
Differences of TNF-α level were both in blood and in sputum in dependence on infectional agent species: in sputum its level was high in H.influenzae exacerbation (290.8±54. pg/ml, p=0.017) as compared with S.pneumoniae (110.6±45.9 pg/ml), M.pneumoniae (85.1±37.1 pg/ml), Ch.pneumoniae (132.6±32.1 pg/ml). While in blood TNF-α level was high in M.pneumoniae (887.2±164.2 pg/ml, p=0.019) as compared with S.pneumoniae (102.2±32.1 pg/ml), H.influenzae (182.2±44.9 pg/ml) and Ch.pneumoniae (495.0±118.3 pg/ml).
Il-8 level was different only in blood. Its level was high in H.influenzae exacerbation (112.0±47. pg/ml. p=0.008) as compared with S.pneumoniae (42.0±13.1 pg/ml), M.pneumoniae (36.2±19.5 pg/ml) and Ch.pneumoniae (53.0±37.2 pg/ml).Proinflammatory cytokines (Il-8, TNF-α) levels in sputum and blood were high at infectional COPD exacerbation.

P3022

Bronchial brush-biopsies cells in dependence on infectional agent species at COPD exacerbation

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Inflammatory process at COPD exacerbation result in high growth activity and injured effects of bronchial epitheliocytes.
To define bronchial brush-biopsies cytogramms peculiarities in dependence on infectional agent species associated with COPD exacerbation.
46 COPD exacerbation patients were examined. Cytological research of brush-biopsies were taken at bronchoscopy was made; the quantitative bacteriological sputum research, definition of diagnostic main IgG, IgM levels to Ch.pneumoniae, M.pneumoniae in serum by means immuno-assay method, in sputum by means of PCR method were made.
Infectional character of COPD exacerbation was confirmed at 36 patients. Typical ciliated epithelial cells count was low in H.influenzae, Ch.pneumoniae, M.pneumoniae 14.6±2.2%, 15.1±2.5%, 15.5±3.0%, ($p<0.05$), than in S.pneumoniae, M.catarrhalis agents (32.7±3.6%, 37.1±5.1%). Reserved cells count was high ($p<0.05$) in M.pneumoniae (12.0±2.7%), than S.pneumoniae, H.influenzae, Ch.pneumoniae, M.catarrhalis agents (5.3±1.1%, 8.4±1.5%, 8.9±1.9%, 5.1±2.3%). Squamous metaplased cells was high ($p<0.05$) in H.influenzae, Ch.pneumoniae, M.pneumoniae (6.3±1.2%, 7.5±1.2%, 7.4±1.6%), than in S.pneumoniae, M.catarrhalis agents (2.5±0.9%, 2.2±0.7%). Dystrophical epithelial cells count was high ($p<0.05$) in H.influenzae, Ch.pneumoniae (56.6±2.1%, 54.9±2.8%), than S.pneumoniae, M.pneumoniae M.catarrhalis agents (46.9±3.0%, 44.7±3.5%, 38.9±4.3%).
Neutrophiles count was high ($p<0.05$) in H.influenzae, Ch.pneumoniae (50.2±4.4%, 56.5±4.2%) than at S.pneumoniae, M.pneumoniae M.catarrhalis agents (36.0±4.0%, 30.8±4.6%, 34.8±4.6%).
H.influenzae, Ch.pneumoniae have more injured effects, but M.pneumoniae result in high growth activity.

P3023

Modification of cell mediated immune-response in patients treated with a polyvalent mechanical bacterial lysate

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Background: Efficacy of Polyvalent Mechanical Bacterial Lysate (PMBL) in inducing the secretion of specific IgA directed to the bacterial antigens administered has been shown.
Aims and objectives: In this double blind placebo controlled study, we analyzed the immunological effect of PMBL, administered to a population of elderly Chronic Obstructive Pulmonary Disease (COPD) patients.
Methods: The treatment provided 1 tablet of PMBL for the first ten days of the month, followed by a 20 day rest. The treatment was repeated for other two months and the follow up was carried out up to six months. Blood cell samples were collected at time 0 (before the beginning of the study), after three months and after six months.
Results: CD4+ and activated T cells increased significantly in treated group, while Treg were significantly reduced. Transitional B cells (in particular T3) were recruited and associated to an increase of early naïve B cells; recruitment of early memory cells was associated to a reduction of "classic" memory B cells. Finally, NK cells were significantly increased in treated patients, while their subpopulations remained unmodified.
Conclusion: In conclusion, PMBL administration causes in COPD patients an important recruitment of cells belonging to the innate immune system, such as NK, a significant activation of early B cell compartments and a clear reduction of regulatory T cells associated to the increase of T cell activation. All these findings confirm that, also in COPD patients, a specific (and also partially polyclonal) activation of B cells occurs, and this seems to be strictly related to the significant clinical results observed.

P3024

Role of endothelial dysfunction, disturbance of haemostatic reactions in pathogenesis of acute exacerbation of chronic obstructive pulmonary disease (AE COPD)

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Purpose: It is to research interrelations of endothelial dysfunction, system inflammatory and haemostatic reactions at patients with AE COPD.
Materials and methods: In open prospective clinical investigation 111 patients with 2 or 3 positive criteria Anthonisen et al. (1987) were included. Men were 91 people (82%), women - 20 people (18%). The smoking index was 37,5±2,06 pack-years. Patients with AE COPD divided on two subgroups: 1st - patients without bronchoectasia, 2nd - patients with bronchoectasia. All patients received therapy by system glucocorticoids. The control group included 35 healthy non-smoking people with middle age 25,9±1,36 years, from them 27 (77,1%) men and 8 (22,9%) women. It was accomplished immuneenzyme definition of CRP, endotelin-1, D-dimers and homocystein maintenance in blood before treatment.
Results: Direct correlation communication between indexes CRP and endotelin-1 (r=0,80, p<0,001), indexes CRP and D-dimers (r=0,65, p<0,01) in the AE COPD was observed. Dynamics of indexes of endothelial dysfunction and thrombogenic risk at patients with various phenotypes AE COPD are in table 1.

Table 1

Indicators	Control group	Patients subgroup 1		Patients subgroup 2	
		before treating	after treating	before treating	after treating
CRP (mg/l)	3.3±0.14	6.5±1.97	4.1±1.16	13.3±3.32*	7.0±1.29*
ET-1 (fmol/ml)	0.3±0.04	1.8±0.55*	1.2±0.38*	2.3±1.08	1.4±0.92
D-dimer (mg/l)	0.1±0.01	1.4±0.34*	0.6±0.12*	1.6±0.45*	1.0±0.26*

*Patients subgroup vs. control group p<0,05.

Conclusion: At patients with AE COPD it is increased levels of D-dimers, which indicates that specifies on thrombogenic and thromboembolic risks.

P3025

Invasive pulmonary aspergillosis in patients with severe COPD. Compative study between probable invasive infection and colonization

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This retrospective study included patients with chronic respiratory disease with aspergillus isolation in respiratory cultures from Jan-07 to Dec-09. The diagnosis of probable invasive pulmonary aspergillus (IPA) was based on compatible clinical setting and HRCT findings. A univariate comparative analysis between IPA probable and aspergillar colonization was carried out.
Results: Of the 103 patients registered 66 patients were excluded. Of the remaining 37 we diagnosed 15 patients (14%) with IPA probable and 22 (21%) as colonization. COPD in 73% of patients (80% GOLD III-IV). The prior use of corticosteroids was higher in IPA, with a greater accumulate mean 405 mgr in IPA v 74 mgr in colonizated ($p<0.05$). A greater number of hospital admissions were detected in IPA. The mean length of hospital stay was higher in IPA with 35±28

days as opposed to 13±22 d. in colonization (p:0.003) and respiratory failure (80% IPA v 36% p:0.009). Antifungal therapy was received by 91% of IPA and by 36% of colonized. The overall mortality rate was 47% in IPA and 23% in colonization. **Conclusions:** 1. Immunosupression by corticosteroids in COPD patients Gold III-IV is the major risk factor for invasive aspergillosis.2. The absence of clinical improvement in correctly treated exacerbations of severe COPD and recurrent isolation of aspergillus in sputum should point to the possibility of the existence of IPA and demand a HRCT scan.3. HRCT findings can detect early IPA in the shape of bronchial spread once bacterial or mycobacterial infections have been ruled out.