128 Supplementation with n-3 PUFA in CF patients: analysis of exhaled breath condensate

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Background: supplementation with n-3 polyunsaturated fatty acids (PUFA) leads to production of leukotriene (LT) B5, which is biologically less active than LTB4. LTB4 is important chemoattractant in neutrophilic inflammation in CF lung disease.
Methods: nine F508del homozygous CF patients (5 females) aged 25.6±3.3 (mean±SD) years were treated for 6 weeks with n-3 PUFA (dosis corresponding to 1.3% of energy intake). Exhaled breath condensates (EBC) were collected before and after treatment. pH and LTB4 concentration (pg/ml) were measured in EBC samples.
Results: pH was increased (from 6.08±0.39 to 6.32±0.18; $p = 0.013$) and LTB4 concentration was decreased (from 227.3±195.0 to 134.9±67.3 pg/ml; $p = 0.075$) in EBC after n-3 PUFA treatment.
Conclusion: LTB4 concentration in EBC tend to be lower after n-3 PUFA treatment and EBC is significantly less acidified, which may reflect less intense neutrophilic inflammation.
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129 Long-term treatment with glucocorticoids in low doses restores affected cytokine production in cystic fibrosis patients

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Plasma levels of cytokines (TNF-α, IFNγ, IL-4, IL-10, TGF-β1) and ACTH of 128 cystic fibrosis (CF) patients and 100 healthy children (HC) have been compared. CF patients were divided into three groups: without anti-inflammatory treatment (WAT Group; $n = 83$), patients treated with alternated course of prednisolone (Pr Group; 0.3–0.5 mg/kg body weight every other day; $n = 16$), and patients treated with azithromycin (Az Group; 500 mg orally three times a week; $n = 37$). The patients of WAT Group demonstrated significant elevation of plasma cytokines including IL-10, IFNγ, and TGF-β1 in comparison with HC (18.2 *vs* 6.8 ng/ml, 262 *vs* 15.6 pg/ml, and 70.7 *vs* 19.6 ng/ml respectively; all $p < 0.001$). It was also noticed a trend to the decrease of TNF-α concentration in WAT Group (10.9 *vs* 17.1 pg/ml; $p = 0.066$). Surprisingly, we did not observe such changes in Pr Group: the cytokine concentrations were not significantly different from those observed in HC. In Az Group the same but not so well-defined tendency has been shown. Thus, although plasma concentrations of TNF-α and IL-10 were not different from corresponding data in HC, IFNγ and TGF-β1 levels remained significantly higher (160.8 *vs* 15.6 pg/ml and 64.5 *vs* 19.6 pg/ml respectively; both $p < 0.005$). It is important to note that CF patients demonstrated low plasma levels of ACTH amounting 4.5 pg/ml in both WAT and Pr Groups and 3.6 pg/ml in Az Groups *vs* 9.2 pg/ml in HC (all $p < 0.05$). These data suggest that the permanent inflammation in CF patients results in hypothalamic-pituitary-adrenal axis underactivity. It seems that prolonged treatment with glucocorticoids or macrolides makes up for stress hormones deficiency.

130 Effect of hypertonic saline on Exhaled Breath Condensate

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Background: Measurement of cytokines in Exhaled Breath Condensate (EBC) has been used in an attempt to qualify the degree of inflammation in the lower respiratory tract. We speculated how inhalation of hypertonic saline (HS) might affect the concentrations of cytokines.
Method: 10 CF-patients with chronic pseudomonas-infection and 10 healthy controls received inhalations of HS (7%), 4 ml. EBC was collected before and immediately after inhalation. Samples were bubbled with argon for 15 minutes to remove CO_2, and pH was measured. Concentration of IL-1β, IL-6, IL-8 and IL-10 were measured using ultra-sensitive ELISA-kits.
Results: Inhalation of HS and EBC-collection was well tolerated by all subjects. We found no difference in pH between or within groups. IL1β decreased significantly in CF-subjects ($p < 0.05$). We found more samples with concentration of IL-6 above detection-level in the control-group ($p < 0.03$). We found no differences between or within groups in concentrations of IL-6, IL-8 or IL-10.
Conclusion: Inhalation of HS and EBC-collection was well tolerated by all subjects. HS induced reduction in IL1β concentration in CF subjects. Studies on larger groups are needed to fully clarify the effect of HS on inflammatory markers in EBC.

Table 1: Measurements on EBC. Results shown as median (range). N equals number of samples with values above detection-level.

	Healthy Controls				CF-Patients			
	Before	N	After	N	Before	N	After	N
pH	6.28 (5.02–7.62)	10/10	6.83 (5.2–7.67)	10/10	6.63 (4.75–7.49)	10/10	6.34 (5.04–7.75)	10/10
IL1β	n.d.	n.d.	n.d.	n.d.	0.45 (0–2.59)	8/10	0.12 (0–0.66)	6/10
IL-6	0.01 (0–0.33)	4/10	0 (0–0.16)	2/10	0.01 (0–0.09)	1/10	0 (0–0.02)	0/10
IL-8	0 (0–0.41)	1/10	0 (0–0.46)	1/10	0.02 (0–0.08)	0/10	0.01 (0–0.52)	1/10
IL-10	0.21 (0–1.53)	5/10	0.41 (0–1.09)	8/10	0.39 (0–0.67)	6/10	0.49 (0–2.04)	7/10

131 Basophil activation test for the early diagnosis of allergic bronchopulmonary aspergillosis (ABPA)

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ABPA is an immunologically mediated lung disease caused by hypersensitivity to Aspergillus antigens. In Cystic Fibrosis (CF) patients ABPA has detrimental effects. Prompt recognition and treatment of this disease is critical for the outcome. Diagnosis is based on a combination of clinical and laboratory criteria (consensus 2003). These criteria are often ambiguous and the use of additional ones would be useful in solving the diagnostic dilemma. Such a criterion could be the positive Basophil Activation Test (BAT), which is the assessment of basophil activation by flow cytometry, after their in vitro stimulation with allergens, using CD63 and CD203 as activation indexes.
The aim of this study was to investigate the diagnostic value of BAT for ABPA in CF patients.
Material and Methods: Using *A. fumigatus* extract as allergen, BAT was applied for 10 healthy controls (Group A) and a total of 18 CF patients: 10 with definite ABPA diagnosis (Group B) and 8 with high level of suspicion for ABPA (Group C). In all the patients serum total IgE and specific IgE for *A. fumigatus* (M3) were determined by fluoro-enzyme-immunoassay (FEIA). Skin brick tests with *A. fumigatus* extract were performed in all 18 patients.
Results and Discussion: Whereas BAT was negative in healthy controls, positive BAT to *A. fumigatus* extract was a constant finding in all Group B patients. Among Group C patients, in a follow up period of 1 year, 2/2 with negative BAT have not developed ABPA, 5/6 with positive BAT have developed the disease, and 1/6 of the same subgroup has had increasing values of total and specific IgE.
Conclusion: Our study points towards a high diagnostic value of BAT for the early recognition of ABPA, though an additional number of patients is needed.