Humoral and cellular immune responses to Blomia tropicalis and its concanavalin A-binding fractions in atopic patients

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Background: Blomia tropicalis (Bt), Dermatophagoides pteronyssinus (Dp) and D. farinae (DF) are the prevalent house dust mites (HDMs). Concanavalin A-binding components derived from B. tropicalis (Bt-ConA extract) have shown to be highly immunogenic in allergic diseases. This study aimed to evaluate the humoral and cellular immune responses to B. tropicalis in sensitized patients.

Methods: A total of 137 patients with allergic rhinitis with/without asthma and 109 non-atopic subjects were selected and analyzed for skin reactivity (SPT), total serum IgE and specific IgE levels to both Bt-total and Bt-ConA extracts, proliferative response and cytokine (IFN-γ and IL-5) production by peripheral blood mononuclear cells stimulated with both extracts.

Results: SPT showed that 70% of patients were sensitized to Bt (Bt+) and no patient was monosensitized to Bt. Similar levels of specific IgE to Bt-total and Bt-ConA extracts were found in Bt+patients, while higher levels of total serum IgE were found in atopic than non-atopic subjects. Significant PBMC proliferation was observed in response to Bt-total extract in Bt-sensitized, but not in Bt-non-sensitized patients and non-atopic subjects, while Bt-ConA extract was able to induce increased proliferative responses in all patient groups. Significant IFN-γ production was observed only after Bt-ConA stimulation in Bt+patients, while Bt-total extract showed no changes. IL-5 production was consistently seen in Bt+patients after allergen-specific stimulation or even with no stimulus.

Conclusion: We can conclude that Bt-ConA extract may contain relevant antigens that are involved in both humoral and cellular immune responses, with potential use in diagnostic procedures.

Inhibitory action of epinastine hydrochloride on eosinophil survival in vitro

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Background: H1 receptor antagonists, so called antihistamine, are accepted to have anti-angiogenesis properties in addition to being H1 antagonists. Epinastine hydrochloride (EP) is the most famous H1 receptor antagonists in Japan and used for treatment of allergic upper airway inflammatory diseases such as polinosis with remarkably success. However, the influence of EP on pro-angiogenesis factor production is not well understood. In the present study, therefore, we investigate the influence of EP on the production of angiogenesis factors, vascular endothelial growth factor (VEGF), keratinocyte-derived chemokine (KC) from murine mast cells in vitro.

Materials and Methods: Murine mast cells were stimulated with ovalbumin in the presence of various doses of EP for 24 h. The levels of vascular endothelial growth factor (VEGF), keratinocyte-derived chemokine (KC) and tumor necrosis factor (TNF)-α levels in culture supernatants were examined by ELISA. mRNA expressions of these factors were also examined by semiquantitative RT-PCR in 4-h cultured mast cells.

Results: EP could suppress the production of VEGF, KC and TNF-α induced by an IgE dependent-mechanisms in dose-dependent manner. The minimum concentration of the agent that caused significant suppression was 45ng/ml. EP also suppressed mRNA expression examined when the agent was added to cell cultures at a dose of 45 ng/ml.

Conclusion: These findings strongly suggest that anti-angiogenesis activity of EP may confer the attenuating effect of the agent on allergic diseases, including allergic polinosis.

Reduced levels of total serum IgE and FcεRI expression in releaser and non-releaser basophils

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434 An Investigation into the interaction of IgE with truncated recombinant CD23 (FcαRII) fragments

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- CD23, low-affinity receptor for IgE, has been widely implicated in the synthesis of IgE as well as in IgE-mediated immune and inflammatory functions. There are two forms of CD23 in humans-CD23a and CD23b, their cell expression and functional activities are different. Structurally, CD23 presents a single membrane-spanning domain followed by an extracellular domain that consists of three regions: the α-helical coiled coil stalk region, which mediates the formation of trimers, followed by the lectin head, which binds IgE, and at the C terminus, a short tail containing an inverse RGD (Arg-Gly-Asp) sequence.

- Aims of the project is to express defined truncated fragments of CD23 and to assess the physical and biological properties of these truncated fragments with regard to: IgE independent mast cell /basophile secretagogue activity. After obtaining all the necessary information concerning the different generation of CD23 fragments as well as the vector PET-14 and pIREs-EGFP, the appropriate primers were designed. The primers are essential for PCR amplification of the desired sequences and for subsequent cloning into the PET-14 and pIREs vectors.

- CD23 a and b-RGD sequence and also the whole things of CD23 a and b have been cloned into pIREs vector for transfection and subsequent evaluation of IgE in functional assays. Overlap PCR was used to produce the different truncated fragments of CD23 molecule, consisting of CD23 a and b minus the RGD sequence (adhersion part).

- In the meantime, the plasmid vectors PET-14 and pIREs were introduced into XL1-E.coli cells in order to be obtained using the Mini and Midi prep protocols (gene transfection). Their DNA and protein was purified and obtained the characterisation by electrophoresis.

- CD23 a and b-RGD sequence and their whole things was transfected into the J558L (mouse myeloma cells) in order to study of expression of EGFP and biological activity by FACS (Fluorescence activated cell sorting) and FACS analysis for investigating the interaction of the IgE with different expression of CD23. Binding of IgE-Fc fragments to cell surface and expressed human CD23 were assessed using flow cytometry to detect the binding of IgE to cell surface receptors, using a biotinylated anti-IgE, followed by a streptavidin phycoerythrin conjugate.

- The results show that there is no expression of CD23 a-RGD sequence on J558L cells and these cells can express CD23 b-RGD sequence but it is not stable.

435 Allergic and hypoallergenic isoforms of the major birch pollen allergen Bet v 1 are differentially uptaken by DCs of allergic and healthy individuals

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- Objective: The present study investigated the different properties of Bet v 1 isoforms to be uptaken by human monocyte-derived DCs (MoDCs) of birch pollen allergic and healthy individuals.

- Methods: Eight birch pollen allergic and 7 healthy donors were included in this study. MoDCs generated from peripheral blood and taken at day 7 of differentiation were incubated with various concentrations of fluorescein isothiocyanate (FITC)-labeled recombinant Bet v 1a, Bet v 1d, and Bet v 1mut for 10 to 120 minutes at 37°C and studied by means of flow cytometry. Mechanisms of antigen internalization were investigated by the same experimental setup in the presence of inhibitors of pinocytosis, endocytosis, and receptor internalization.

- Results: FITC-labeled proteins were taken up by MoDCs in a dose-and time dependent manner. The percentage of FITC positive cells increased from 1 to 5 μg/ml of labeled proteins whereas the cells internalized most proteins between 10 and 30 minutes. At the lowest concentration of 1 μg/ml, the uptake of Bet v 1a was lower compared to the uptakes of Bet v 1d and Bet v 1mut in DCs of both study groups. This fact did not change for healthy individuals when higher concentrations of proteins were used whereas in allergic individuals Bet v 1a uptake at higher concentrations was similar to that of the two other isoforms. Inhibition of the uptake was generally lower using DCs from allergic compared to those of healthy individuals.

- Conclusion: All different agents showed inhibitions indicating that the uptake of Bet v 1 isoforms is not only mediated via one pathway but an interplay of mechanisms like receptor-mediated internalization, endocytic processing and macropinocytosis. Our observations implicate that different mechanisms are involved in Bet v 1a uptake in allergic individuals possibly leading to the induction of signals for Th2 polarization. This study was supported by the SFB F1802 of the Austria Science Fund and the Austrian Academy of Sciences.
Purpose: It is known that polymorphism of the human leukocyte antigen (HLA) class II can restrict specific IgE responses. We investigated whether particular HLA-DRB1 polymorphisms contribute to the development of AD and EW sensitization in Korean children with AD.

Methods: A total of 185 patients with AD and 109 normal controls (NC) with no personal and family history of allergy were included. HLA-DRB1 typing was done using PCR-SSO (sequence specific oligonucleotide) and PCR-SSCP (single strand conformation polymorphism) methods. Phenotype frequencies of the HLA-DRB1 alleles of AD patients were compared with those of NC. AD patients with allergy to EW was defined as group A (96 patients) whose EW specific IgE was over 2 kU/L in less than 2 year old age and over 7 kU/L in greater than 2 year old age. Group B (89 patients) was defined that EW specific IgE was negative among AD patients. Phenotype frequencies of the HLA-DRB1 alleles in group A and group B were compared with those of NC. HLA-DRB1 alleles were classified into functional groups (A, De, Dr, E, Q, R, a) and frequencies of functional groups in group A and group B were compared with those of NC.

Results: HLA-DRB1*1101 was present at significantly higher frequency in AD patients compared with NC (12.4% vs 1.8%, P=0.002, OR=7.796, CI 1.775-35.883) and was regarded as a factor associated with AD susceptibility. The result was significant after Bonferroni correction (Pc=0.048). The frequency of HLA-DRB1*0803 (10.8% vs 19.3%, P=0.043) was decreased in AD compared with NC, showing a weak protective effect against the development of AD. HLA-DRB1*0802 was decreased in group A compared with group B (2.1% vs 10.1%, P=0.021) and was regarded as a weak protective factor against the development of egg allergy in AD. HLA-DRB1*1501 was increased in group A compared with group B (22.9% vs 11.2%, P=0.036) and was regarded as a weak susceptibility factor associated with the development of egg allergy in AD. HLA-DRB1 functional group “a”, in which DRB1*1501 is included, was also weakly associated with the development of egg allergy in AD. However, none of theses results remained significant after Bonferroni correction.

Conclusion: There was a significant association between HLA-DRB1*1101 and AD. Weak association between HLA-DRB1*1501 with susceptibility to and HLA-DRB1*0802 with protection against the development of EW allergy in AD were observed.

438 Probiotic components can induce the Th1 polarization through keratinocytes

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Background: Probiotics are defined as live non-pathogenic microorganisms that beneficially affect the host. It has been suggested that administration of probiotics may have therapeutic and/or preventive benefits by improving the Th1 responses in atopic dermatitis (AD).

Aim: We investigated the possibilities that skin can be used as a target for the prevention of AD and Lactobacillus rhamnosus, one of the most commonly used in the therapeutic intestinal probiotics, can induce the Th1 responses through keratinocytes.

Methods: Keratinocytes were treated by L. rhamnosus for 2 hours and then cultured with new media for 24 hours. The supernatant (KCM) of L. rhamnosus-treated keratinocytes were collected and used for the maturation of immature dendritic cells from 5 normal and 5 AD individuals. CD14+ cells and naive T cells were isolated from peripheral blood of normal and AD individuals using isolation kits. CD14+ cells were differentiated to immature dendritic cells (iDCs) with GM-CSF (200 ng/ml) and IL-4 (20 ng/ml) for 2 hours and then matured with proinflammatory cytokines (IL-1β, TNFα) for 7 days. The iDCs were activated by KCM or LPS lipopolysaccharide (LPS) (positive control) for 48 hours and cocultured with naive T cells for 7 days.

Results: We confirmed the maturation of iDCs by KCM and LPS through morphological changes and the expression of mature DC specific markers (CD80, CD83, and CD86) in normal and AD individuals. ELISA analysis showed that the mature DCs activated by KCM could induce the Th1 responses. So, the IFN-γ was significantly increased in the co-cultured supernatants of mature DC and naive T cells, but IL-4 level was almost basal. Furthermore, we found that IL-8 and HBD-3 were highly increased in keratinocytes after L. rhamnosus treatment.

Conclusion: Probiotics, L. rhamnosus, can improve the Th1 responses through keratinocytes. The effects of probiotics will be mediated by IL-8 and/
or HBD-2 secreted by keratinocytes. However, there is no difference for the effects of probiotics between normal and AD individuals.

439 Interaction of aeroallergens with the respiratory interphase: degradation, processing and transmission via the epithelial barrier

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Background: Antigen presenting cells are considered as the most important immune cells giving rise to allergy or tolerance development. But APCs are already embedded in a cytokine milieu, which in part predicts the immune response. Epithelial cells form a barrier to the environment and they are activated upon contact with molecules e.g. allergens. Therefore, they might play a pivotal role in the sensitization process. We studied the fate of the major grass pollen allergen Phl p 1 and its molecular modifications after contact with the respiratory interface.

Methods: The influence of mucosal secretions (nasal secretion, bronchial lavage fluid) was investigated by immunoblotting and zymography and the uptake and transmission of the allergens by epithelial cells (cell lines and nasal and lung biopsies) were studied by FACS analysis, determination of cytokine release and immunohistology.

Results: Nasal secretions of individuals suffering from bronchial inflammation or allergy to aeroallergens showed proteolytic activity. Experiments using supernatants from mast cells or neutrophils, similar to acute inflammation, resulted in a partial cleavage of the allergens. To investigate the allergen uptake two epithelial cell lines were used mimicking different sections of the lung, A549 (derived from alveolar pneumocytes) and Calu-3 (from the upper respiratory epithelium). Both cell lines were activated by Phl p 1 as demonstrated by the release of IL-8 and IL-6. Calu-3 cells in contrast to A549 cells expressed MHCII, a prerequisite for antigen processing and presentation. A549 cells, on the other hand, take up allergens by macropinocytosis and probably perform transcytosis. First results of human lung slices incubated with Phl p 1 showed only a faint allergen uptake, while macrophages in the alveolar space showed a considerable uptake. Whether macrophages after allergen uptake enter the interstitium again and/or whether the epithelial cells transfer allergens or fragments to professional APCs in the interstitium is still under investigation.

Conclusion: The secretions of the respiratory tract cause a more or less incomplete fragmentation of allergens which may facilitate the allergen uptake by epithelial cells. Our results reveal differences in the uptake and transmission of allergens in the upper and lower airways. While the epithelial cells of the upper part probably degrade and process the allergens, cells of the lower part seem to transmit the unprocessed allergens.

440 The level of IL-6 and its soluble receptor at children with atopic march

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The estimation of influence of various programs of therapy on system and local level IL-6 and SR-IL-6 was made at development atopic march of teenager patients. The research allowed to reveal, that therapy with the help of topical steroids practically did not influence SR-IL-6 level in serum of blood (22,72±0,25ng/ml), but led to decrease in IL-6 level at patients with BA (0,17±0,001 pg/ml) and combination BA and AR (3,59±0,37 pg/ml). At combination of BA and AR the simultaneous decrease of IL-6 and SR-IL-6 was established while using topical steroids. On the contrary, having clinical BA and AD evidence steroid therapy led to one-stage increase cytokine (6,45±0,41pg/ml), and level of receptor to it (31,76±0,15ng/ml). Thus, research of nasal lavage can be offered as noninvasive method for the control of efficiency of therapy of patients having combination BA and AR while using topical steroids. Therapy with topical steroids of patients with BA and with combination BA with AR and AD provided decrease of IL-6 level and did not lead to change of SR-IL-6 level in serum of blood. Therapy with topical steroids did not lead to decrease in content of IL-6 both in serum and in nasal secret only in group of patients with combination BA and AD.

441 Systems biology of type-I birch pollen allergy in human patients

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Previous work in type-I pollen allergies has focused on lymphocytes and immune responses. Here we begin to analyse with a systems biology top-down view the differences in nasal epithelium obtained from healthy and allergic subjects. Light and immunoelectron microscopic analysis showed that birch pollen Bet v 1 allergen bound to epithelial cell surfaces within minutes even during non-symptomatic winter seasons only in allergic, but not healthy individuals. Bet v 1 also travelled through epithelium together with lipid rafts/caveolae and reached mast cells only in allergic, but not healthy individuals within minutes. A putative viral entry protein E3 ubiquitin-protein ligase and three enzymes involved in lipid rafts/caveolae metabolism were among the few Bet v 1 binding proteins found in allergic subjects with affinity chromatography and LC-MS2. Nasal epithelial cell transcriptomics during non-symptomatic winter season identified a list of putative receptors by which Bet v 1 might be recognized. It also suggested that defence mechanisms (such as expression of histatins) could be impaired in allergic patients. Comparing nasal epithelial transcriptomics taken in winter and during symptomatic summer seasons provided hints to the cellular perturbations enabling the Bet v1 traffic through nasal epithelial cells and tissues. Thus application of discovery and hypothesis driven methodologies on human nasal epithelial tissue could provide new hypothesis worth further analysis of the underlying molecular mechanisms.

The most significant aspect of this and other top-down explorative studies using whole genome or other large-scale analysis is that they can provide truly new hypothesis. Before this work few would have argued that the nasal epithelium is so markedly different in healthy compared to allergic subjects already during non-symptomatic winter session. Now based on these validated results the mechanisms of Bet v 1 pollen allergen binding to and traffic through the epithelium can be further explored and analysed. Furthermore it must be noted that no single data domain, whether is would have microscopy, proteomics, transcriptomics etc. would have alone pointed out the above-described phenomenon. Thus systems level understanding of complex pathophysiological phenomena will need all possible wet
442 Maternal tolerance achieved before pregnancy is transferred to the offspring and prevents asthma development in the next generation

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In this study we hypothesized that immunologic tolerance acquired before conception can be transferred from the dam to the pup. In a murine model, we induced tolerance before conception by oral application of antigen. We then immunized the offspring of tolerized dams with the same antigen. While the offspring of naive dams developed an asthma-like phenotype with airway hyperreactivity, inflammation, Th2-cytokine production after immunization, the offspring of tolerized dams was protected, even when immunized as late as 8 months after birth. Critically involved in tolerance transfer is allergen-specific IgG that was increased during pregnancy in the tolerized mouse, fetus and newborn. FcRn⁺/⁺ mice, that cannot transport IgG via the placenta, transferred tolerance to the offspring only when the missing diaplacental IgG transfer was compensated by IgG transfer via breast milk from tolerant dams but not when the offspring was crossfostered by naive mice. Inhibition of IFN-γ, produced by memory B cells in the offspring, abrogated the protective effect of maternal tolerance demonstrating its crucial memory role in materno-fetal tolerance transfer. Our data show that maternal immunologic memory has significant and persistent impact on the immune response of the offspring indicating that e.g. allergy prevention strategies might be effective for more than one generation.

443 Meditation as a suggestion for allergies and autoimmune diseases

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Objective: The aim of the present study was to analyze the effects of a Meditation program on Complete Blood Cell count(CBC), IgG and C3 as a marker of specific and nonspecific humoral immunity.

Methods: 78 subjects, aged 18–56 years, of whom 62 were male and 20 female undergoing a period of one month Meditation, were excluded. They were all experiencing medication for the first time. Before starting the meditation training, all of these volunteers have been visited by a physician and were all experiencing meditation for the first time. They were all healthy subjects(78 subjects), not taking any type of drug and with regular life habits were chosen to be at this clinical trial. Blood samples were taken from all subjects, the day before the study commenced, and again one month later, at the end of the study. The blood parameters investigated included the number of Leukocytes(total Leukocytes, Monocytes, Neutrophils, Eosinophils, Basophils, Lymphocytes),Red cell count(RBC), Hemoglobin(Hb), Hematocrit(Hct), Mean cell volume(MCV), Mean cell hemoglobin (MCH), Mean cell hemoglobin concentration(MCHC), Red cell distribution width(RDW),Platelet count, as well as the concentrations of immunoglobulins(IgG) and complement(C3). 4 of these volunteers has been excluded from research plan because of their sickness during the period.

Results: Statistically significant differences were found between first and second blood samples of volunteers showing lower numbers of total Leukocytes(=0.02) and Eosinophil(=0.01),number and percentage of Monocytes(=0.01),as well as complement C3 concentration(=0.004).

Conclusion: These findings demonstrate that one month of practicing Meditation, can decrease some immunological parameters. According to these results Meditation can be suggested for Hypersensitivities(Allergies and Autoimmune Diseases) although further studies seem to be needed.

Key words: Meditation, Complete Blood Cell count(CBC), IgG, C3.

444 ASTHMA TREATMENT

444 A randomized, double-blind, placebo controlled trial on the effect of zinc supplementation on bronchial asthma as measured by sputum eosinophil count and asthma control test (ACT) in children

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Background: Zinc being a major dietary anti-oxidant has a protective role in the airway epithelium. It may therefore have important implications for asthma where the physical barrier is vulnerable and compromised.

Objective: We investigated the effects of zinc supplementation on bronchial asthma as measured by sputum eosinophil levels and asthma control test (ACT) in children.

Methodology: There were sixty-six asthmatic subjects age range 7 to 18 years old were randomized to receive zinc 20mg/day(n=29) or placebo (n=37). Sputum eosinophil count were checked before supplementation and 12 weeks later. Likewise asthma control test (ACT) scores were obtained before supplemetations and at 4 weeks, 8 weeks and 12 weeks thereafter.

Results: After 12 weeks of supplementation, the sputum eosinophil count decreased in both groups but the zinc group has the more significant change in eosinophils as compared to placebo at p=0.029 and p=0.059 respectively. However, ACT score from week 1 to week 12 showed no difference between the zinc and placebo group. (p=0.069).

Conclusion: A dietary zinc supplementation significantly reduced sputum eosinophil count as compared to placebo. However, the decline in sputum eosinophil count was not associated with improvement in asthma control. Zinc should be considered in decreasing airway inflammation but not asthma control.

445 Documentation of the relative efficacy and safety profile of the salbutamol delivery by slow infusion(SI) VS inhalation(IN) to patients with acute severe bronchial asthma

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Purpose: Patients with acute severe bronchial asthma are in a moribund position. The art of devising an effective delivery system which may be followed by an optimal relief in the degree of bronchospasm is the goal of resolving the imminent urgency of an acute severe bronchial Asthma.

Methods: Patient’s both sex age16-50 years, with history of acute severe bronchial asthma have been included in the study. They had been Group A, medicated with Prednisolon 15–20 mg immediately followed by 10 mg 6hourly for 2 days along with Salbutamol 4mg/kg body weight in slow infusion, Group B, having being medicated with Prednisolon as in Group A, along with inhalational delivery of Salbutamol in dilution of 1.5ml+2.5ml and 1.25ml+2ml (for adult and childhood bronchial asthma respectively). Therapeutic response as observed had been as under.

Results: Group B, had been followed with optimal bronchodilation in about 5–10 minute while with group A, it was 15–20 minutes.

Conclusion: Group B had therapeutic advantages vs A (e.g.) optimal bronchodilation in a relatively shorter period, no hazard of parenteral administration and a simple technique of delivery that can be managed even by any one.

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Clinical Implication: Both techniques of delivery of Salbutamol are effective with in the limits of availability of services of healthy care providers and appropriate selection of cases.

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Role of body weight reduction by diet control and physical exercise in controlling asthmatics (children and adults) with obesity
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The prevalence of asthma has increased worldwide since the 1960s. The incidence of asthma is high among children; however, a relative high annual incidence is also estimated in adults. Studies for risk factors have mainly analyzed cross-sectional design data based on prevalent cases of asthma. Such studies may determine either cause, consequence or both. Incidence studies have mostly focused on occupational asthma or smoking habits. Since the late 1990s, obesity has been reported to be associated with asthma, and an increase in the prevalence of obesity has been reported along with a parallel increase in asthma prevalence. However, a convincing relationship between asthma and obesity has not been established. Accordingly, this study was carried out aiming at demonstrating the effect of weight reduction on clinical, functional and serological parameters in obese asthmatics. In this study, obese asthmatic patients showed marked improvement clinically, functionally, with marked decrease in number of exacerbations and medications used.

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Feasibility and applicability of secondary prevention of asthma in allergy practice
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Background: The possibility for secondary prevention of asthma was highlighted by the prevention of asthma study, which demonstrated that immunotherapy in patients with allergic rhinitis, could prevent asthma in some patients. The feasibility of secondary prevention of asthma and the proportion of patients likely to be benefited need to be demonstrated as immunotherapy requires time to exert its beneficial effects.

Methodology: All the patients who underwent a detailed evaluation including allergy testing during the period 2003 to 2006 were included in the study. A structured questionnaire was applied to collect data on demography, a detailed clinical history, the duration of asthma and allergic rhinitis and the proportion of patients whose rhinitis preceded asthma and the time interval between the development of rhinitis and asthma.

Results: A total of 934 cases were included in the study. 701 (75%) patients had allergic rhinitis for varying intervals before they developed asthma. 143(15.5%) patients continued to have rhinitis for more than 5 years without developing asthma. 576(66%) patients had only asthma without rhinitis. 33(3.5%) patients developed asthma before they developed rhinitis.

327 (46.6%) developed asthma within 1 year of developing rhinitis. Of these, 164 developed asthma within 6 months and 169 between 6–12 months. 374 patients developed asthma more than a year after the onset of rhinitis and the frequency gradually decreased with increase in duration of rhinitis.

Conclusion: Majority of patients had rhinitis before they developed asthma. Nearly 50% of these patients with preceding rhinitis developed asthma within one year, who may not be amenable for secondary prevention with immunotherapy due to the delays in referral to the allergist and the time required for obtaining the necessary benefit from immunotherapy. Patients who develop asthma alone or develop rhinitis after the onset of asthma are not candidates for secondary prevention. Of the total cases studied, less than 40% can undergo interventions targeted towards secondary prevention of asthma. Intense education of the Family physicians and ENT specialists is needed to ensure prompt referral to the allergist to help these patients.

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Asthma control assessed by the asthma control test and markers of airway inflammation
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Aim: to assess the relationship between ACT and different markers of airway inflammation in asthmatic subjects.

Methods: In a crossover design, we studied 106 patient suffering from mild or moderate asthma (median age 31 years IQR 16–45). Fifty five percent were only treated with short beta agonist on demand. On the same visit patients fulfilled the ACT questionnaire, exhaled nitric oxide (eNO) (NiOx Mini) was measured and bronchial challenge with hypertonic saline and sputum induction were performed.

Results: On the whole sample ACT was modestly correlated with eNO (r=−0.4, p<0.001), eosinophils in induced sputum (r=−0.30, p<0.05) and the slope of hypertonic saline bronchial challenge (r=0.43, p<0.001). Patients with very good control (ACT 24 or 25) had significantly lower levels of eNO (41 vs 72 ppb, p=0.001) and eosinophils (10 vs 6%, p<0.05). Correlations of all these variables improved, when only the 55 patients treated with beta agonist on demand were included: eNO (r=−0.55, p=0.0001), eosinophils in induced sputum, (r=−0.50, p=0.002) and slope of hypertonic saline bronchial challenge (r=0.52, p=0.0001). ACT was not correlated to FEV1/FVC.

Conclusion: asthma control assessed by the Asthma Control Test was correlated to direct and indirect markers of airway inflammation. ACT and pulmonary function variables were not correlated.

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Salbutamol metered-dose inhalation is as effective as nebulisation in managing acute asthma exacerbations in hospitalized children in accordance with an asthma pathway
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Background: Acute asthma exacerbations have traditionally been managed with nebulised bronchodilators in the emergency rooms as well as for inpatients. In 2003, Singapore was hit by SARS and the fear that the use of nebulisation could cause the spread of the virus prompted the development of an asthma pathway using MDI.

Methods: A 19-item questionnaire was administered to all attending A&E for acute asthma in the KK Women's and Children's Hospital. The questionnaire was anonymous and voluntary and assessed the effect of nebulisation and MDI on patient management and the organisation of asthma care. A total of 121 questionnaires were collected from 2004 to 2005 from 1241 patients presenting with acute asthma.

Results: Ninety one per cent (91%) of patients preferred MDI. Ninety five per cent (95%) of emergency staff preferred MDI. Ninety nine per cent (99%) of nurses, 94% of residents and 93% of registrars preferred MDI.

Conclusion: Salbutamol metered-dose inhalation is as effective as nebulisation in managing acute asthma exacerbations in hospitalized children in accordance with an asthma pathway.
Aim: To compare the length of stay in children admitted for asthma exacerbations before and after the introduction of an asthma pathway for managing acute exacerbations with salbutamol metered-dose inhalers (MDI) instead of nebulisation.

Methods: Children presenting to the Children’s Emergency and admitted to the wards for asthma exacerbations were managed according to an asthma pathway.

Results: There was no increase in admissions from the emergency department or to high dependency or to the intensive care after implementation of the asthma pathway with the use of Salbutamol MDI instead of nebulisation. The average length of stay decreased from a high of 2.78 days pre-implementation of pathway to a low of 2.19 days post-implementation. The average cost per patient decreased from $1,136.85 in 2001 to between $974.00–$1,011.05 post pathway implementation.

Conclusion: The asthma pathway has shown that the use of salbutamol MDI is as effective as nebulisers in the management of acute asthma exacerbations and has led to a decrease in the average length of hospitalisation as well as decreased cost per patient.

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A troublesome diagnosis of Churg Strauss Syndrome

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**Case Report:** A 41-year-old asian man was admitted to the emergency room of our hospital with fever, wheezing, dyspnea, arthralgias.

His past history was remarkable for allergic rhinitis and recurrent episodes of asthma since his arrival in Italy at the age of 34.

The patient was treated with a course of systemic corticosteroids (80mg daily prednisolone), theophylline, inhaled budesonide/formoterol 160/4.5 mcg (twice a day), montelukast 10 mg. His chest radiograph and full blood count including eosinophil count were normal. His spirometric test showed FEV1 1.57 (42%), FVC 2.52 (57%), PEF 240 l/min.

One month later he was seen in the outpatients department and noted to be generally well, very wheezy and 2 days before the visit he has noticed the comparison of hemoptysis.

His chest radiograph and full blood count, including eosinophil count, were normal. Antinuclear antibody and antineutrophil cytoplasmic antibody (ANCA) were both negative. Sputum culture and urine microscopy were unremarkable. Aspergillus precipitins and radioallergosorbent test (RAST) against Aspergillus were negative. Cultures for mycobacteria were negative. The electrocardiogram and echocardiogram were both normal.

Computed tomography of the paranasal sinuses dimostrated a near-total opacity with the material of the soft parts of the normal sinus and right sphenoidal sinus, as well as of ethmoidal cell, the right maxillary sinus and nasal cavities. Thorax computed tomography revealed generalized enlargement of the mediastinic lymph nodes.

Treatment with montelukast was stopped and high dose oral prednisolone was commenced.

The patient elected to discontinue his inhaled treatment. Three months later his symptoms of asthma returned. Chest-X-ray showed patchy bilateral infiltrates in the lungs. He had marked hypereosinophilia (15%), elevated serum IgE levels (465 IU/ml), and antineutrophil cytoplasmic antibody (ANCA) were positive. His spirometric test showed FEV1 1.60 (45%), FVC 2.35 (55%), PEF 240 l/min. He required a further short course of oral corticosteroids.

His symptoms remained troublesome and his treatment was changed to cyclosporine 3 mg/kg and his condition showed marked clinical as well as radiological improvement.

**Discussion:** We must carefully diagnose and treat patients with middle-age onset of severe asthma, because the symptom may be a lung manifestation of CSS, in which various organs are involved as a result of systemic necrotizing vasculitis.

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Educational courses improve asthma management and treatment

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**Background:** Education of the patients is one of the first goals of asthma management in all the international treatment protocols. We made this study in order to estimate the need and the effect of an educational program prepared by the Macedonian Asthma Center.

**Methods:** We studied 300 asthmatics. We prepared a pilot educational course, covering the most important and “hot” subjects concerning asthma, following the guidelines from the Global strategy for asthma management and prevention. (May 1996), and a questionnaire with 10 questions treating the problems concerning the treatment problems in asthma management. The questions were grouped in 2 parts: I - general knowledge of asthma medications and the necessity of adequate and continuous anti-inflammatory treatment; and II - accent on the emergency interventions and actions during acute exacerbations. The questionnaire was completed by all of the patients, before and after attending the educational course.

**Results:** Before visiting the course, only 46.3% answered correctly the questionnaire (39.9% correct answers on part I and 52.8% on part II). These results imply that more than 50% of the tested asthmatic population do not realize the need for preventive medication or would in fact take the wrong medication during worsening of their disease. After visiting the educational course the results are as follows: 54.8% overall correct answers (nonsignificant-NS). The results from part I showed that only 52.4% (NS) of the patients gave correct answers and 73.6% (significant) answered the II part correctly.

**Conclusion:** We conclude that patients more willingly accept advice on emergency self management of acute asthma exacerbations, but it is the most difficult to overcome the “fear of preventive treatment” and to change the dogmatic opinion on the therapeutic issues of asthma management. The study shows the necessity of organizing further and continuous asthma educational programs on all levels of the health care.

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Clinical evaluation of severe asthma attack requiring tracheal intubation and respirator management

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**Background:** Recently, the number of patients requiring hospitalization because of an asthma attack has decreased; however, there are still patients who require hospitalization for tracheal intubation and respirator management for a severe asthma attack. Therefore, we evaluated the background features of 20 asthmatic patients who required tracheal intubation and respirator management in our hospital.

**Methods:** We evaluated 20 asthmatic patients who visited our hospital from January 2001 to December 2005 and required tracheal intubation and respirator management. All the patients had severely exacerbated asthma, as determined on the basis of the guideline of the Global Initiative for Asthma (GINA) 2006, and they required tracheal intubation and respirator management. We evaluated their history of smoking, the days from asthma attack...
onset to their visit to our hospital, the level of asthma control as determined from the GINA 2006 guideline, treatments taken before the patients visited our hospital, their frequency of visiting a hospital, the reason for tracheal intubation and respirator management based on the Asthma Prevention and Management Japanese Guideline 2006, and their prognosis.

Results: The patients who required tracheal intubation and respirator management were the following: 1. smokers, 2. patients not taking or irregularly taking treatment, 3. patients who used inhaled short-acting β2 agonist only at the time of an asthma attack, and 4. patients not using inhaled glucocorticosteroids. The reasons for tracheal intubation and respirator management were as follows: PaO2 of less than 50mmHg despite maximum oxygen administration in 30% of the patients; sudden increase in PaCO2 leading to unconsciousness in 20%; both PaO2 of less than 50mmHg despite maximum oxygen administration and sudden increase in PaCO2 leading to unconsciousness in 25%; and severe ventilatory or cardiorespiratory disturbance in 25%.

Conclusion: We observed that a thorough education of patients and treatment mainly using inhaled glucocorticosteroids are important for preventing a severe asthma attack that requires tracheal intubation and respirator management.

Methods: 30 patients with asthma and AR, aged 7–17 years were randomly allocated. During eight weeks, 20 of them received BDP-CFC (at least 500 mcg/day) inhaled exclusively by the nose (mouth closed) using a large-volume (650 ml) spacer attached to a facemask. Control group (10 subjects) received conventional treatment, i.e., BDP by dual administration (aqueous intranasal and oral inhalation through the mouthpiece of the same spacer device). Every two weeks a clinical score for AR and peak expiratory flow (PEF) was assessed by independent observers. Spirometry was performed at admission and at the end of the follow up. A minimum decrease in 66% in clinical scoring was considered as therapeutic success.

Results: Therapeutic success rate was of 50.0% for the experimental group and of 70.0% for the control group (p=0.11). PEF and FEVI weren’t statistically different in the two groups neither at admission nor by the end of the treatment (p > 0.10).

Conclusion: Results suggest that this alternative treatment could be recommended for the simultaneous treatment of AR and asthma, specially in developing world. Other advantages are higher compliance, lower costs and fewer side effects.

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Assessment of quality of life in caregivers of asthmatic children
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Background: Interest in the impact of illness on day to day function is leading investigators to include both disease specific and generic health related quality of life (HR QOL) questionnaires in a broad range of clinical studies and to gain a full picture of the impact of asthma on the lives of caregivers of asthmatic children, it is necessary to make direct measurement of health related quality of life.

Methods: In response to this need, we used Juniper’s Pediatric Asthma Caregiver’s Quality of Life Questionnaire (PACQLQ) that has been developed based on guidelines for construction of over a dozen validated disease specific quality of life instruments. The PACQLQ that contains 13 items in two domains of emotional and activities disturbances. The study design consisted of an 18 month single cohort study. Patients participating in the study were 113 children, 7–17 years of age, with a wide range of asthma severity and their caregivers. For each caregiver a PACQLQ was completed. One week before visit patients recorded morning peak flow rates, medication use and symptoms in a diary. After complete physical examination, for determining of asthma severity, spirometry was performed.

Results: The questionnaires after statically analysis showed good levels of both longitudinal and cross sectional correlations with the conventional asthma indices and with general quality of life.

Conclusion: We found a good relevancy between severity of asthma and QOL scores and more disturbances of QOL in caregivers of male asthmatic patients than caregivers of female asthmatic patients. Bronchial asthma as a chronic and devastating disease can affect not only life style of the patients but also their caregivers. Increment of our knowledge about these disturbances can help the physicians for better understanding of burdens of their patients.

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Unified treatment in school children with allergic rhinitis and asthma
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Aim: To verify the efficacy of beclomethasone dipropionate (BDP), administered through nasal inhalation, in the simultaneous treatment of asthma and allergic rhinitis (AR).

Materials and Methods: The thirty patients with severe BA and concomitant the AR were prescribed to intranasal mometazon spray in a dose of 100 microgram two times a day for a one month. This treatment followed after two weeks course of standard hospital therapy that includes steroids (intravenous, inhalation), b2-agonists, etc. The age of the patients was ranged from 25 to 50 years old. Therapy effectiveness was controlled both by clinical and by laboratorial methods: spirography and daily pickflowmetry, determination of the resistance of a bronchial tree, rhinomanometry and identification of ECP.

Results: All patients at the end of the first month of treatment mentioned the stable improvement of nasal breathing and sleep recovery. Also, a valid reduction of the resistance of nasal apertures was discovered (p<0.005). The majority of the patients (93.3%) have the improvement of clinical picture of the BA, which confined in less amount of asthma attacks and also improvement of the functional and laboratorial measures: PEF growth, decrease of its variability (p<0.05), reduction in the resistance of the bronchial tree (p<0.05) and normalization of ECP.

Conclusion: Conducted research proved a valid increase in the effectiveness of supporting therapy of the BA with the combination of active treatment of the AR, which becomes apparent besides clinical improvement in an additional growth of measures of ventilating capability of lungs, level of the resistance of the bronchial tree and nasal apertures and normalization of ECP content.

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Influence of topical intranasal steroids on improvement of ventilating capability of lungs in patients with bronchial asthma
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Background: Treatment of a bronchial asthma (BA) is often conducted without an allowance of a concomitant allergic rhinitis (AR), which can be a reason of unsuccessful results in an achievement of a maximized therapeutic effect.

Aim: To determine the degree of influence of allergic inflammation of the upper respiratory tracts on ventilating capability of lungs in patients with bronchial asthma.

Methods: 30 patients with severe BA and concomitant the AR were prescribed to intranasal mometazon spray in a dose of 100 microgram two times a day for a one month. This treatment followed after two weeks course of standard hospital therapy that includes steroids (intravenous, inhalation), b2-agonists, etc. The age of the patients was ranged from 25 to 50 years old. Therapy effectiveness was controlled both by clinical and by laboratorial methods: spirography and daily pickflowmetry, determination of the resistance of a bronchial tree, rhinomanometry and identification of ECP.

Results: All patients at the end of the first month of treatment mentioned the stable improvement of nasal breathing and sleep recovery. Also, a valid reduction of the resistance of nasal apertures was discovered (p<0.005). The majority of the patients (93.3%) have the improvement of clinical picture of the BA, which confined in less amount of asthma attacks and also improvement of the functional and laboratorial measures: PEF growth, decrease of its variability (p<0.05), reduction in the resistance of the bronchial tree (p<0.05) and normalization of ECP.

Conclusion: Conducted research proved a valid increase in the effectiveness of supporting therapy of the BA with the combination of active treatment of the AR, which becomes apparent besides clinical improvement in an additional growth of measures of ventilating capability of lungs, level of the resistance of the bronchial tree and nasal apertures and normalization of ECP content.

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An all Wales audit of secondary care of acute asthma during February 2006
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We audited the secondary care offered to acute asthmatic patients admitted to all 17 hospitals in Wales, UK during February 2006. The audit analysed referral source, care delivered by the ambulance service, emergency units, medical admission units and inpatient units in all cases of asthma aged 18 years or greater. All data was extracted by qualified medical personnel. The results show that 248 patients were admitted during that month (36 patients were excluded as not having asthma). 44 were acute severe/severe, 91 moderate, 75 mild and in 38 severity was not documented. Ambulance transfer was used in 99 cases, 63 patients calling the ambulance directly. Overall, ambulance staff measured vital signs well but peak flow (PFR) was measured only 8 cases. Most patients received nebulised bronchodilators/high flow oxygen in the transfer process but steroids were used rarely and observations were repeated in only 27 cases. Emergency units were the primary site of acute care. PFR was measured in only 67% of cases compared to 90% having oxygen saturations measured. Only 162 of 248 cases had a record of steroid administration and only 67% were reassessed. Of those admitted (99) only 51% were under specialist care and only 32% were treated in a specialist respiratory ward. Overall discharge planning both from emergency and inpatient units was disappointing with only 13% of inpatients having written management plans and only 4.5% of patients discharged directly from emergency units offered further review.

Secondary care of acute asthma in Wales does not confirm to national guidelines for acceptable levels of care.

Conclusion: The awareness and recognition of childhood asthma, the use of peak flow meter, the implementation of GINA guidelines and the use of ICS has improved among the doctors of the cities in China. Nevertheless, dissemination of the GINA program among non-specialists and doctors in countries and communities must be continued and re-enforced.

458 Impact of patient education on knowledge, attitude, practice and self-efficacy in patients with asthma in India

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Background: Patient related factors like poor knowledge, individual beliefs and attitudes of patients influence control of asthma leading to nonadherence. This study was conducted to develop a validated KAPSE questionnaire for the Indian population and to assess the knowledge, attitude, practices and self-efficacy (KAPSE) of patients in Mysore regarding asthma and the impact of patient education on KAPSE in these patients.

Methodology: A total of 225 adult patients participated in the KAPSE study and were part of the study that compared the efficacy and quality of life in patients receiving beclometasone, budesonide or fluticasone. A questionnaire with 22 items was selected from Knowledge, Attitude and Self-Efficacy (KASE), a validated instrument for asthma and suitably modified to suit the cultural and educational level of the population. Two medical experts performed content validation and only those items with content validation index of above 0.75 were retained. Cognitive debriefing in 5 patients confirmed the suitability and acceptability of the questionnaire. Patient counseling individually and in groups and information leaflets about the pathology of asthma, drugs and delivery systems were conducted at every visit on 7 occasions, until 6 months. KAPSE was assessed at baseline before education and at the end of the study.

Results: Patient education significantly improved most of the questions assessing Knowledge, Attitude, Practice and Self-efficacy compared to the baseline (p<0.05). Subgroup analysis revealed that KAPSE at baseline were not influenced by Age, Gender and Duration of disease (p>0.05), but was related to educational levels and area of residence (p<0.05). Following patient education KAPSE of all the patients improved significantly (p<0.05) and there was no difference between patients of different areas of residence.

Conclusion: This study demonstrated that regular patient education significantly improves different aspects of Knowledge, Attitude Practice and Self-efficacy of patients regardless of their age, gender, education or place of residence, which could positively influence patient medication adherence.

459 Asthma - a curable disease

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Background: In normal individuals, the Serum Histamine Binding Capacity (SHBC) is 20 to 30% whereas it is only 0 to 5% in allergic patients. The SHBC can be raised with Histaglobulin (HG) if it is given after preparing the patient properly and drugs like mast cell stabilisers, bronchodilators, antihistamines and anti-inflammatory drugs are given along with HG therapy. In allergic patients serum IgE level is high which comes down after HG therapy and medicines. Patient becomes symptom free and cured.

Methods: It was an observational study for 14 years. 161 patients were registered at different periods from out-patients consisting of all age-group, both sexes and from urban and rural areas. Immunoglobulin E (IgE) levels were estimated and other routine tests were done before starting treatment.
Patients were prepared before HG therapy; HG was given in two phases: Phase I—6 primary doses, Phase II—3 booster doses. Along with HG therapy, drugs like mast-cell stabilisers, and H1 receptor-specific Histamine antagonists (Loratadine, Rupatadine, Cromolyn etc.) bronchodilators (Theophylline), antihistamines (Cetirizine, Levocetirizine) and anti-inflammatory drugs (Monteleukast) were given. 2 to 3 months after 3rd booster dose IgE levels were estimated for 61 patients. Patients were examined periodically to assess clinical improvement.

Results: Out of 161 patients, 152 patients (94.6%) are free from asthma and allergic rhinitis symptoms. Among 152, 96 patients are free for more than 4 years. 9 patients did not respond satisfactorily. Out of 61 patients, 55 patients (90.2%, p<0.0001) showed reduction in IgE level and are clinically free from asthma. 6 patients showed raised IgE level and correspondingly no clinical improvement. It is also found out that allergic manifestations are lowest up to 2 years of age; between 2–5 yrs, it increased and more males are affected; the occurrence rate came down in the age group of 6–15 yrs in both sexes. Above 15yrs, again occurrence rate raised and more females were affected.

Conclusion: Thus in our study comprising of 161 patients having the complaints of allergic asthma with or without allergic rhinitis, the HG therapy given after preparing the patient and continuing the above-mentioned drugs, during HG therapy was found to be effective in curing the allergic asthma. HG is effective in raising the SHBC thereby improving the immunity of the patient and also in reducing the serum IgE level.