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4th International Drug Hypersensitivity Meeting
Rome, 22-25 April 2010

Epidemiology

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4th International Drug Hypersensitivity Meeting
Rome, 22-25 April 2010

Epidemiology

Severe cutaneous adverse drug reactions in Singapore

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Poster

Introduction: Adverse drug reactions are common in hospitalized patients. Though, the majority of cutaneous reactions are mild and self-limiting, a minority of patients developed severe cutaneous adverse drug reactions such as Stevens-Johnson syndrome, toxic epidermal necrolysis and drug rash with eosinophilia and systemic symptoms. There have been few studies examining the presentation and outcome of such severe adverse reactions in Singapore. *Methods:* The clinical and laboratory data of all inpatients dermatology consults with a diagnosis of severe cutaneous adverse drug reaction were retrospectively analyzed over a 1-year period. The diagnosis of cutaneous adverse drug reactions were made based on clinical features, exclusion of alternative causes, supported by ancillary investigations such as histological and laboratory findings. The initial drug causality was derived and ranked based on a consideration of composite factors such as temporal relationship between the drug ingestion and onset of drug reaction, known epidemiological risk, improvement on withdrawal, and exclusion of other causes. *Results:* Over the 1 year study period, there were a total of 731 inpatient dermatology consultations of which 97 patients were diagnosed with cutaneous adverse drug reactions. Among these patients, there were 35 with severe cutaneous adverse drug reactions this made up 36.1% of all referrals for cutaneous adverse drug reactions. The clinical presentations included: Stevens Johnson syndrome (4 patients), Stevens-Johnson syndrome-Toxic epidermal necrolysis overlap (3 patients), Toxic epidermal necrolysis (7 patients), Drug rash with eosinophilia and systemic symptoms (18 patients) and acute generalized exanthematous pustulosis (3 patients). Among the Stevens-Johnson and toxic epidermal necrolysis spectrum, the likely putative drugs consisted of antibiotics (4 patients), Anti-convulsants (3 patients), Allopurinol (2 patients), Non-steroidal antiinflammatory drugs (1 patient) and other drugs (3 patients). Among the patients with DRESS, the putative drugs consisted of allopurinol (6 patients), Anti-convulsant (6 patients), Antibiotics (3 patients) and others (2 patients), The causative drugs for AGEP included cephalosporin, enalapril and phenytoin (1 patient each). There were a total of 5 deaths; 2 were patients with SJS/TEN overlap and the other 3 patients had toxic epidermal necrolysis. *Conclusions*: Cutaneous adverse drug reaction is a common dermatological problem in hospitalized patients and a significant proportion of patients may present with severe reactions such as Stevens-Johnson syndrome, Toxic epidermal necrolysis, Drug rash with eosinophilia and systemic symptoms as well as acute generalized exanthematous pustulosis. Our data suggests that the causative medications and prognosis of patients with severe cutaneous adverse drug reactions in Singapore is similar to those in other parts of the world.

Anti-epileptics induced severe cutaneous adverse drugs reactions (SCARs): a clinical characteristics, risk factors and complication study of cases in Taiwan

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Poster

Antiepileptic drugs have been reported to be associated with severe cutaneous adverse drug reactions (SCARs) including SJS, TEN and DRESS. To investigate the characteristics of severe cutaneous drug eruption caused by epileptics in Taiwan, we conducted a perspective study of patients with SJS, TEN and DRESS caused by antiepileptic drugs (AEDs). In addition, we also followed the subsequent antiepileptic treatments in patients diagnosed with AEDs-SCARs to clarify the cross-sensitivity of aromatic-structure AEDs. All the hospitalized patients diagnosed as SJS, SJS-TEN overlap, TEN and DRESS caused by AEDs from January 2003 to June 2009 at Chang Gung Memorial Hospital in Taiwan were enrolled into this study. Clinical courses, culprit drugs (carbamazepine, phenytoin, lamotrigine, phenobarbital, and oxcarbazepine), latent period, organ involvement and complications, and the mortality were analyzed. The organs involvement and laboratory data were compared between AEDs-SJS/TEN and AEDs-DRESS. We also elucidated possible risk factors related to the severity of disease(s), including age, dose/titration rates, and co-medication. Based on our analysis, we

found that carbamazepine is the most common causative drug for SJS/TEN and phenytoin is the most common causative drug for DRESS in Taiwan. Organs involvements, especially liver function impairment, were more common seen is AEDs-DRESS than in AEDs-SJS/TEN. The mortality rate of AED related SJS or TEN was 6% (5 of 83 SJS/TEN cases) and mostly caused by phenytoin (4 phenytoin and 1 carbamazepine). Only one case died from AEDs-DRESS and was caused by lamotrigine. By following up AEDs usage after patients with AEDs-SCARs, most of patients were well tolerant to non-aromatic-structure AEDs, e.g. valproate and topiramate, and only one case of oxcarbazepine-DRESS was cross reacted to lamotrigine.

Hypersensitivity syndrome to dapsone: a systematic review

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Poster

Dapsone (4,4-diaminodiphenylsulfone) is indicated for the treatment of leprosy, for prophylaxis of Pneumocystis pneumonia (PCP) in HIV-patients, malaria prophylaxis and also frequently used in chronic inflammatory conditions such as dermatitis herpetiformis. Obligatory adverse drug reactions (ADR) of this sulfone are hemolytic anemia and methemoglobinemia whereas the hypersensitivity syndrome ([HS]; synonymous with sulfone syndrome) is a rare, but potentially fatal ADR. To date, a systematic appraisal of the literature concerning clinically important information about HS such as incidence, characteristics of HS, and prognostic factors is missing. We performed a systematic review on all published cases with HS in the literature from 1949 to October 2009. A standardized literature search in MedLine and Embase supplemented by hand research identified 115 publications (98 case reports, 17 epidemiologic studies) totalling 343 patients with HS (age 5-83 years, 60,1% male). Regarding the global distribution of reported HS cases, most patients originated from Asia (73,2%), followed by Europe (12,2%) and other continents (Australia and Africa each 3,8%, North and South America each 3,5%). With 71,8% leprosy was the most prevalent condition for treatment with dapsone (n=309). Further entities were chronic inflammatory dermatoses (20,1%) and prophylactic use (PCP 2,9%, malaria 2,6%). Based on 12 epidemiologic studies the estimated prevalence of HS is 1,4% (126/8798 patients receiving dapsone). Latency between initiation of dapsone administration and onset of HS ranged between 6 hours and 21 weeks (weighted mean 30,25 days; n=263). The complete HS, i.e. all of the four cardinal symptoms fever, skin rash, hepatitis and lymphadenopathy, presented 62,3% of all reported patients (n=252). The related therapy of HS in all cases (n=258) consisted of withdrawal of dapsone, whereas in 12% withdrawal definitely proceeded not directly after the onset of HS. Glucocorticosteroids were administered in 81,1% (n=212). 89,7% of the HS patients recovered completely (n=343). In the 35 patients who died, hepatic failure was the most frequent cause of death (18 cases). Based on published data, HS to dapsone may not be as rare as expected, the recovery rate is 89,7% and the fatality rate is 10.3.

European Network for Cutaneous Adverse Effects of Drugs (ENCAD)

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Poster

Background: GA²LEN, the Global Allergy and Asthma European Network, has been created in the sixth framework program in order to ensure integration and harmonization of research and clinical care of all areas of allergy. In the field of drug allergy GA²LEN is cooperating with ENDA in a general and in a specific project (GALEN-DA). The Department of Dermatology and Allergy, Charité, Berlin, Germany as one of the partners of GA²LEN has longstanding interest and experience in consultant services for doctors in the field of cutaneous drug reactions. Based on this experience and the knowledge that non-dermatologists very often have problems with the classifications of the cutaneous signs of drug allergy, the project ENCAD has been created in close discussions with GA²LEN. It is now funded by the EU Regional Development Funds. *Materials and Methods:* ENCAD will develop systematic guidelines for taking pictures of patients with drug rashes and then offers real-time dermatological conferences for the discussion of cases and suggestions in clinical and diagnostical approaches for patients. In addition to clinical features it is possible within the service to give an expert opinion on histological findings or even as an extension of service to offer derma-histological examination and evaluation of skin biopsies sent in by the individual collaborators. Already now, online consulting service for histology is established. This offers partners equipped with high resolution video microscopes to send real time pictures of specimens to our department. This saves time of usual mailing of samples and allows interactive discussions during microscopy. *Results and Conclusion:* The ultimate ambitious research aim of ENCAD is to develop a computer-based algorithm to allow a probability ranking of the eliciting drugs. This is of highest importance since in frequent cases patients are taking more than one drug and the eliciting agent is not easy for the treating physicians to detect.

Hypersensitivity to acetaminophen or celecoxib in patients with aspirin/NSAIDs intolerance

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Poster

Background: Acetaminophen and celecoxib (selective COX-2 inhibitor) are known as relatively safe therapeutic alternatives for patients with aspirin/NSAIDs intolerance. However, cross-reactivity among these drugs and risk factors for cross-reactivity have been poorly understood. To find the cross-reactivity of aspirin and aceta-

minophen/celecoxib, and to determine risk factors for intolerance to acetaminophen or celecoxib in patients with aspirin intolerance. *Materials and Methods:* We performed a retrospective study of patients of Seoul National University Hospital and Seoul National University Bundang Hospital from November 2003 to February 2009. The medical records of 96 aspirin intolerant patients who experienced acetaminophen(and/or celecoxib) oral provocation test were reviewed. Aspirin intolerance was defined as positive responses in challenge to aspirin (N=86) or convincing clinical history of aspirin and/or other NSAIDs hypersensitivity (N=10). *Results and conclusion:* Among 96 patients, 89 experienced oral acetaminophen provocation test, and 22 (25%) reacted to acetaminophen. History of chronic urticaria (p=0.008, OR 3.79, 95% CI 1.36 ~ 10.52) and nasal polyp (p=0.002, OR=14.38, 95% CI 2.04 ~ 101.40) were statistically associated with a higher risk of acetaminophen hypersensitivity. Sixty-nine celecoxib provocation tests were done, and 6 (8.7%) were positive to celecoxib challenge. No demographic and clinical characteristics were associated with a higher prevalence of reactivity against celecoxib. Acetaminophen and celecoxib may not be tolerated in up to 25% and 8.7% of aspirin intolerant patients respectively. Nasal polyp as well as history of chronic urticaria seems to represent relevant risk factors for intolerance to acetaminophen in patients with aspirin hypersensitivity.

Hypersensitivity reactions to drugs: a retrospective analysis of clinical characteristics of patients consulting for suspected drug hypersensitivity in an Allergy Centre in Norway

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Poster

Introduction: Drug hypersensitivity (DH) constitutes one of the most frequent reasons for consultations in allergology services with an increasing prevalence in recent years. There are few true epidemiological data on DH. *Objectives:* To describe the clinical characteristics of patients with suspected DH. *Methods:* The medical records of 20 consecutive patients with suspected DH, enrolled from a large retrospective study designed to include about 400 patients consulted in a Norwegian Allergy Centre, were investigated with respect to history, skin tests and serology. *Results:* Mean age was 43.25 years (range 12-84), and 70% were females. 13 patients (65%) had a previous allergic history, including pollen allergy and/or allergy to other allergens, 8 of them (62%) had also a previous reaction to drugs. The drugs most often suspected were NSAIDs (65%), antibiotics (40%), and paracetamol (25%). 53.8% of those who had reactions to NSAIDs had also reactions to antibiotics and/or paracetamol. Cutaneous symptoms (37.5% urticaria) were most frequently reported (60%). Quincke's edema, respiratory, gastrointestinal and circulatory symptoms accounted for 50%, 35%, 35% and 30% of the symptoms, respectively. Anaphylaxis was reported in 10 patients, and 45% of the reactions occurred within 1 hour after taking the drug. Total serum IgE was increased (> 120 KU/L) in 40% and serum ECP was increased (> 22.0 μg/L) in 20% of patients. 3 patients had antigen-specific IgE antibody concentration above 0.35 KU/L (2 to penicillin and 1 to morphine/pholcodine). Skin prick tests (SPT) were performed with suspected drugs in 19 patients. Only 1 patient (5.3%) had a positive SPT to morphine/codeine, 17 (89.5%) had negative results and 1 patient had inconclusive SPT. Open oral provocation tests (PT) with suspected drugs were performed in 7 patients of whom only 1 patient had a delayed reaction to penicillin, the remainder was negative. *Conclusions:* Suspected DH reactions occur most frequently in female patients, and in those who have a previous history of allergic reactions. The most common manifestations are cutaneous symptoms, but life-threatening reactions may occur. NSAIDs and antibiotics are the two drug families most frequently suspected. These preliminary results are based on a limited number of patients and may not be extrapolated to the general population. PT needs to be included in diagnostic protocols in order to evaluate suspected DH reactions.

Ophthalmologic sequelae after Stevens-Johnson syndrome and toxic epidermal necrolysis

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Poster

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are acute life-threatening muco-cutaneous reactions associated with a high morbidity and mortality. Among survivors long-lasting sequelae are known but not analyzed in a prospective study. Especially ophthalmologic problems after SJS/TEN may be long-lasting and impairing the patient's daily life substantially. The population-based registry on severe skin reactions in Germany (dZh) ascertains cases of SJS and TEN since 1990. All cases are validated by an independent expert committee separating between definite, probable, possible and no cases of SJS/TEN. In the framework of the European Registry of severe cutaneous adverse reactions to drugs and collection of biological samples (RegiSCAR), a cohort of patients was followed for more than one year. In the acute stage of the disease they were seen by an investigator of the dZh and a follow-up visit took place 8 (+/- 2 weeks) later. One year after SJS/TEN surviving patients received a questionnaire including specific questions on ocular problems. In a period of three years (01.03.2003 - 28.02.2006) 228 patients with SJS (128), SJS/TEN-overlap (73) and TEN (27) were included in the analysis and stratified according to their eye-involvement. Severe eye-involvement in the acute stage of the disease was defined as severe conjunctivitis and severe blepharitis diagnosed by an ophthalmologist, mild eye-involvement was ocular redness and pain. Severe ocular sequelae were conjunctival and corneal erosions and vision loss, whereas multiple symptoms were categorized as mild sequelae by ophthalmologists. 45% (104/228) of the patients had severe eye-involvement during the acute stage of the disease without a difference between SJS and TEN. After 8 weeks 70% (50/72) of available patients still had eye-problems, 26% of them severe ones. Milder symptoms most frequently reported are loss of eye-lashes, substantial dryness and photophobia. 66% (38/58) of patients with eye-sequelae one year after SJS/TEN suffered from those already at the 8-week-follow-up, whereas in 20 patients eye-problems seem to have occurred later. Independent of the first occurrence dryness, photophobia and tearing are the predominant long-lasting symptoms. These findings suggest that long-term ophthalmologic follow-up is

needed for all patients with SJS/TEN even if they had only mild ocular symptoms during the acute stage of the disease and several weeks later.

Prevalence of aspirin intolerance in patients with asthma in Turkey: A cross-sectional survey

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Poster

Background: Limited data reported that the prevalence of aspirin intolerant asthma (AIA) in adult asthmatic patients ranges from 1% to 20%, and these differences attribute to the populations assessed and study methods used. There is no well design study specifically focused on AIA in Turkish asthmatic patients, therefore we aimed to assessed the prevalence of AIA in adult patients with asthma. *Material and Methods:* This was a prospective, national and multicenter study. A structured questionnaire was administered to patients with asthma via face-to-face interview by a pulmonologist and/or an allergist at 7 centers across Turkey throughout 2006-2007. *Results:* A total of 1344 patients with asthma (F/M: 1081/263: 80.5%/19.5%, mean age: 45, 7±14.2 years) were consequently enrolled in the study. Of all patients, 856 (66.6%) had controlled, 276 (21.4%) had partially controlled, and 154 (12%) had uncontrolled asthma. Atopy rate was 47%. Prevalences of allergic rhinitis, rhino sinusitis, and nasal polyposis were 49%, 69%, 20%, respectively. There were 270 patients with a history of nasal polyposis. Of them, 171 (63.3%) reported previous nasal polypectomy and 49.7% of them had a history of more than two nasal polypectomy. Aspirin intolerance was reported in 149 (11.2%) asthmatic patients. In addition to aspirin, 55, 4% of patients reported reaction to other nonsteroidal anti-inflammatory drugs, 5, 6% to COX-2 inhibitors, and 11,3% to antibiotics in AIA group. The diagnosis of aspirin intolerance was based on reliable history in 108 cases (76.6%), and oral aspirin provocation was performed in 31 (22%) patients. Clinical presentations of aspirin intolerance were respiratory in 64%, respiratory and dermatological in 15%, only dermatological in 12%, and systemic in 9% of the patients. In the comparison of patients with AIA and aspirin tolerant asthma, AIA was significantly associated with nasal polyposis, rhino sinusitis, family history of aspirin hypersensitivity, history of reactions to other nonsteroidal anti-inflammatory drugs, COX-2 inhibitors and antibiotics, the use of systemic steroid and LTRA, emergency department visits and hospitalization due to asthma during the past year ($p< 0.001$, (OR: 4,943, 95% CI:3,010-8,117) were independent predictors for AIA. *Conclusion:* This cross-sectional survey showed that AIA is highly prevalent among adult asthmatics in our country and its prevalence seems to be affected by family history of aspirin intolerance and presence of nasal polyps.

Epidemiology of NSAID hypersensitivtiy

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Poster

Background: Nowadays adverse drug reactions (=ADR) represent an increasing problem. Non-steroidal anti-inflammatory drugs (=NSAID) are frequently used analgesics and can often cause ADRs. Although the majority of these ADRs are mild and confined only to the skin, NSAID can also induce severe and life-threatening reactions. These reactions are in most cases interpreted as pseudo-allergic, presumably non immunologic, but their dynamics and appearance in a subgroup of patients is suggestive for the involvement of an IgE-mediated mechanism. *Methods:* In this study, we retrospectively analysed data of 501 patients from our outpatient clinic population of the past seven years with drug hypersensitivity reactions in the history after using NSAID. Data was evaluated regarding the culprit drug or drugs, type and severity of reactions, age, gender, atopy, number of co-medication, co-morbidity and infections etc. as risk factors. Further, the skin test and provocation test results were reviewed for their clinical relevance and reliability. The type of ADR caused by NSAID and the time interval between drug intake and the appearance of the first ADR-symptoms were evaluated. *Results:* Acetylsalicylic acid (ASA), paracetamol, diclofenac, mefenamic acid and propyphenazone were named as top five causative drugs for ADR in the case histories. The most common symptoms were angioedema, urticaria, pruritus, exanthema and dyspnea. ASA caused dyspnea, angioedema and urticaria in the majority of the cases. Diclofenac was found to be the most common culprit for severe anaphylactic reactions, followed by paracetamol and propyphenazone. 60% of the NSAID reactors suffered from an atopic disease or had an atopic predisposition. Although 74% of the patients were women, there was no significant difference in gender regarding a proven or strongly suspected ADR. There was a significant association between proven hypersensitivity reactions and reaction initiation after drug intake regarding the time interval. *Conclusions:* Our data suggest that -atopic predisposition is a risk factor for intolerance reaction to NSAID, -ASS accounts for non-immunologic, intolerance reactions, whereas severe anaphylactic reactions to diclofenac and/or propyphenazone seem to be IgE-mediated, -a shorter time interval between drug intake and appearance of symptoms is supportive for clinical relevance and could be an indicator for IgE-mediated ADR.

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Generalized bullous fixed drug eruption: analysis of 62 cases

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Poster

Generalized bullous fixed drug eruption (GBFDE) is an adverse reaction of the skin presenting with nummular, sometimes larger erythematous, often brownish-violaceous macules or patches. Between the lesions, normal skin can be seen, on which Nikolsky sign is negative. Sometimes mucus membranes are slightly affected with blisters or erosions. The patients with GBFDE rarely have fever or malaises. Some patients may experience GBFDE more than once and recurrent episodes may lead to more extensive skin detachment resembling toxic epidermal necrolysis (TEN). The histopathology of both conditions reveals subepidermal blistering and necrotic keratinocytes of the blister roof. GBFDE and TEN were separated by Kauppinen in 1972 based on their clinical appearance and by results of oral provocation test. The population-based registry on severe skin reactions in Germany (dZh) ascertains cases of Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) since 1990. All cases are validated by an independent expert committee separating between definite, probable, possible and no cases of SJS/TEN. Potential cases notified to the registry also include many cases of GBFDE that were first considered to be TEN. 64 cases of severe GBFDE were validated of which 62 patients with relevant clinical and demographic data could be analyzed. 3 of them were children, whereas the average age of 59 adults was 75.6 years. 34 patients were male, 28 female. The overall condition of the patients was good, but approx. 30% were in a reduced state. Fever occurred in 57% of the patients with a mean temperature of 39°C. The mean percentage of blisters related to the body surface area (BSA) was 9%. The extent of erythematous lesions related to the BSA was 20% with a preponderance of extremities followed by trunk, buttocks and face. In 77% mucus membranes were affected with a predominance of genitalia in both men (58%) and women (48%). In 62% of the patients prior events could be identified. Most frequently associated drugs were cotrimoxazole (50%), analgesics such as paracetamol (20%) and metamizole (18%). The overall mortality within 6 weeks after the onset of the adverse reaction was 21% (13/61); all but one patient with lethal outcome were above 70 years. Compared to the published data on TEN, patients with GBFDE are significantly older and the mortality rate is lower. However, patients with repeated and/or extensive GBFDE of more than 70 years have a substantial risk for lethal outcome.

Hypersensitivity reactions to diclofenac – just another NSAID?

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Poster

Background: Diclofenac (DCF) is associated with a high incidence of adverse reactions, some of which are thought to be immune mediated. Some patients experience hypersensitivity reactions only to this NSAID and some on the first administration. The aim of this study was to determine the prevalence and the characteristics of hypersensitivity reactions to DCF notified by our Drug Allergy Unit (DAU) to the Portuguese Pharmacovigillance System (INFARMED), in order to compare them with the remaining NSAIDs reactions. Methods: From all adverse reactions to NSAIDS notified to INFARMED between January 2001 and December 2009, we selected those notified by our DAU, particularly involving DCF. Measures of descriptive statistics were used for analysis. *Results:* In this period INFARMED received 336 notifications on adverse reactions to NSAIDs, 51 (15%) to DCF. Our DAU was responsible for 27% (91/336) of all notifications to NSAIDs and 55% (28/51) to DCF. Diclofenac was the most frequent NSAID responsible for drug adverse reactions reported to INFARMED and studied in our DAU, followed by nimesulide, ibuprofen and etoricoxib. Analysing only DCF hypersensitivity reactions reported by our DAU (n=28), there was no differences in gender (M/F=1/1) and the mean age of patients was 46 y (SD of 4). Nine patients (32%) were single reactors to NSAIDs. Sixty one percent of patients (n=17) had immediate reactions (<1h). Most of the patients (46%) had mucocutaneous symptoms. The same pattern was noticed on reactions to the remaining NSAIDs. Forty three percent of patients (n=12/28) had anaphylaxis (Sampson et al, JACI 2006, criteria). Only 6 patients previously tolerated DCF. This information was unknown in the remaining patients. Skin tests were performed on 10 patients with immediate reactions - 2 positive IDT at 1/10th (2,5 mg/mL) concentration and 4 at 1/1. One patient had anaphylaxis during this procedure. Four of the 6 patients with positive IDT were single reactors with history of anaphylaxis. Oral challenge with alternative NSAID (meloxicam/etoricoxib) was performed in all patients with no immediate or late reactions. *Comments:* In Portugal, DCF is responsible for most of the serious reactions regarding NSAIDs. Anaphylaxis is a frequent and severe clinical presentation of reaction to this drug. Positive IDT in single reactors with immediate reactions, can suggest a possible immunological underlying mechanism.

Stevens-Johnson syndrome/toxic epidermal necrolysis: are prescribing physicians correctly informed on the risk?

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Poster

Background: Stevens-Johnson Syndrome/toxic epidermal necrolysis (SJS/TEN) are severe cutaneous adverse reactions associated with multiple incapacitating sequelae and a high mortality. We evaluated the relevance of the mention of these adverse events in drug reference dictionaries used by prescribing physicians, compared to the best available evidence provided by 2 case-control studies (1,2). SCAR and EuroSCAR have highlighted different classes of drugs based on their risk of inducing SJS/TEN: "high risk", "lower but definite risk" and "no established risk". *Materials and Methods:* Systematic analysis of drug dictionaries for the mention and the degree of a risk of inducing SJS/TEN for: all drugs with «high risk » (15 generic names), several drugs with "lower but definite risk" (5) and several widely used drugs with "no established risk" (12). Drug dictionaries (2009 editions) from Austria, France, Germany, Netherlands and United

Kingdom were used. *Results:* 2300 drug descriptions were evaluated. Discrepancies were found between the countries, and between the various descriptions for drugs containing the same active ingredient. Most of the 323 drug descriptions, associated with a "high risk" of inducing SJS/TEN, mention the risk, except for one French drug description of phenytoin and 4 Austrian drug descriptions of allopurinol. For the 614 drug descriptions associated with a "lower but definite risk", 6% of them do not mention the warning in drug dictionaries. Finally, one third of drugs with no established risk contains a warning for SJS/TEN or a partial warning (e.g. bullous cutaneous eruption). The frequency of the risk also varies for identical generic substances. *Conclusion:* The majority of drugs associated to a high risk of SJS/TEN have a warning concerning this adverse event in drug dictionaries. 6% of drugs with "lower but definite risk" do not mention it. Conversely, SJS/TEN is over-reported among drugs with "no established risk". Better information on the risk of severe adverse drug reactions in drug dictionaries is essential for prescribing physicians, for the quality of drug assessment in pharmacovigilance, and also for legal issues.

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Chronic ocular complications in patients with Stevens-Johnson Syndrome and toxic epidermal necrolysis

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Poster

Background: Inflammation and epithelial erosion of the ocular surface often occur during the acute stage of Stevens-Johnson syndrome (SJS) and its more severe variant, toxic epidermal necrolysis (TEN), leading to ocular complications and scarring in the chronic stage. Severe ocular surface disease arising from SJS and TEN encompasses a spectrum of ocular manifestations and complications that is often associated with significant visual morbidity. We clarified the characteristics of chronic ocular complications in SJS and TEN. *Materials and methods:* Ninety-four patients with a confirmed history of SJS or TEN and chronic ocular complications were included. The results of a detailed ophthalmic examination of each patient were recorded on an itemized data-collection form. Complications were categorized as corneal- (superficial punctate keratopathy, epithelial defect, loss of the palisades of Vogt (POV), conjunctivalization, neovascularization, opacification, and keratinization), conjunctival- (hyperemia and symblepharon formation), and eyelid complications (trichiasis, mucocutaneous junction involvement, meibomian gland involvement, and punctal damage), and these 13 components were graded on a scale from 0 to 3 according to their severity. A structured interview and/or examination of the patients' medical records addressing clinical manifestations at disease onset were also administered. *Results:* Patient age ranged from 1 to 83 years (mean age: 41.6 ± 18.5 years; mean ± SD). At disease onset, the age of the patients ranged from 0 to 77 years (mean age: 26.2 ± 18.8 years), and the duration of the illness ranged from 1 to 48 years (mean duration period: 16.1 ± 15.2 years). Visual acuity in 97 of the 186 eyes (52.2%) was worse than 20/200. Only 34 eyes (18.3%) had good visual acuity of 20/20 or better. The complication that most severely affected the patients was the loss of the POV, suggesting the loss of corneal epithelial stem cells. All 13 complications were significantly correlated with visual acuity. Common cold-like symptoms preceded skin eruptions in 75 patients. Extremely high fever (above 39° C) was reported by 86 patients. Acute conjunctivitis and oral involvements (blisters, erosions, and bleeding of the mouth and lips) occurred in all patients who could recollect their symptoms in detail. Fingernail loss at the acute stage or deformation at present existed in all patients. *Conclusions:* Visual dysfunctions that accompany chronic ocular complications in SJS and TEN are serious and prolong throughout the life of the patient. Common cold-like symptoms and acute conjunctivitis followed by skin eruptions with high fever can signal the initial indication of these diseases. Ocular, oral, and fingernail manifestation might be essential for the diagnosis of SJS and TEN with ocular involvement.

Drug allergy history record in out-patient prescriptions

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Poster

Background: Drug allergy is an immune mediated-adverse drug reaction. The drug allergy history is important information for prevention of drug allergy re-occurrence. As a result, the Good Prescribing Practice Policy (GPPP) of Ramathibodi Hospital which is a large university hospital, Thailand was established in order to promote drug allergy history record by physicians in prescriptions. However, the consequence of GPPP has not been investigated. For this reason, the objectives of the present study were to determine the rate and characteristics of drug allergy history record in out-patient prescriptions. *Materials and Methods:* Prescriptions from nine out-patient departments were retrospectively sampled every 3 months during October 2006 to June 2007. Drug allergy history record by physician in prescriptions was assessed by pharmacy student. The drug allergy history record was categorized by drug name and clinical manifestations. The manifestation was verified whether it was true allergic reaction. All data were analyzed by descriptive statistics. *Results:* Of total 3,300 prescriptions, drug allergy history information was not specified at all in 2,089 prescriptions (68.9%). No drug allergy history was recorded in 599 prescriptions (19.8%) while drug allergy history was recorded in 342 prescriptions (11.3%). Of these, 430 medications were specified. Both drug name and clinical manifestations were recorded for 389 medications (90.5%) out of 430 medications but only 51.4% were verified as true allergic reactions. Antimicrobials (54.0%), NSAIDs (7.4%), ACEIs (5.1%), and aspirin (3.3%) were mostly recorded as medicines that caused allergy history record. Two top ranking antimicrobial medications were penicillins (23.0%) and sulfonamides (17.0%) out of 232 medications. *Conclusion:* The rate of drug allergy history record in prescriptions was rather low. Almost half of the recorded information was still not valid. Promotion of the GPPP on valid drug allergy history is essential.

Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS): Results from the RegiSCAR

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Oral communication

Background: Cases of a drug induced hypersensitivity syndrome are reported under various names, especially DIHS (drug induced hypersensitivity syndrome) and DRESS (drug reaction with eosinophilia and systemic symptoms). Case definition for this reaction pattern however is regularly poorly applied, sometimes resulting in overlap with other severe cutaneous adverse drug reactions (SCAR). *Patients and methods:* RegiSCAR, a multi-national registry of SCAR, enrolled 201 potential cases of DRESS from February 2003 to July 2009. Using a standardized scoring system (1) and blinded for risk factors, including drug exposure, 117 cases were validated as probable or definite DRESS. *Results:* Gender distribution showed 51 males (44%) and 66 females (56%). Females were significantly younger (median age 41.5 vs. 57 years, p=0.03). The reaction generally started with fever, sore throat and exanthema. All cases showed exanthema, slight mucosal involvement was found in 56% of cases. Fever ≥38.50C was present in 89%, and lymphadenopathy in 54%. Involvement of a single internal organ (36%) or variable combinations of up to 5 internal organs (55%) was frequent (91%) and predominantly concerned liver (75%), kidney (36%), lung (32%), spleen (15%), and muscle/heart (13%). Eosinophilia was documented in 95%, and atypical lymphocytes in 67%. In all but 3 patients the duration of the reaction was protracted (≥15 days). Drug causality was very plausible in most cases (88%). Antiepileptic drugs, especially carbamazepine which was prescribed for various indications, were involved in 35% of cases, followed by allopurinol in 18%, while other medications, often reported as inducing DRESS, were less frequently found. The median time lapse after drug intake was 28 ± 17 days for all drugs with "very probable" or "probable" causality. During the acute phase, 2/117 patients died. *Conclusion:* This series confirmed the variable clinical picture of DRESS, highlighting fever, lymphadenopathy, skin, hematological and visceral involvement. Original findings were female predominance, a significant younger age of females, a high prevalence of mucosal symptoms, and a lower mortality than generally stated. The ranking of associated medications was likely less biased than in many prior reports. Clinical characteristics, co-morbidities, timing of the reaction and indications for causative medications differed sufficiently from the profile of other SCARs to support that DRESS is an original phenotype.

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Risk factors for immediate and late hypersensitivity

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Poster

Rationale: Drug Hypersensitivity reactions (DHRs) are imputed in 6-10% significant morbidity, mortality and socioeconomic costs. Some risk factors having an important role in drug hypersensitivity. The purpose of this retrospective hypersensitivity reactions. *Methods:* Between January 2005 and July 2009, we studied 530 patients them underwent standardized diagnosis questionnaire suggested by ENDA and late hypersensitivity reactions according to the EAACI position statement. *Results:* Women were prevalent in both groups, but 17% of patient from age in the immediate reactions group. Non-steroid anti-inflammatory drugs immediate reactions, while sulfonamides, anticonvulsant drugs besides NSAIDs more than 3 drugs taken regularly at the time of the reaction in late manifestations of atopy, auto-immune and cardiovascular diseases, and chronic HIV infection and malignancy were related to late reactions. Familiar history 7% of late reactions. *Conclusions:* Different risk factors appear to be implicated in susceptibility of risk factors for drug hypersensitivity requires well designed, large, prospective, stratification of risk factors may help on drug hypersensitivity prevention.

Epidemiology of hypersensitivity reactions to penicillins in Slovenia

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Poster

Background: Penicillin antibiotics are still one of the most common drug families involved in allergic drug reactions. Many patients are referred as penicillin allergic, what is not so often confirmed. Diagnostic procedure consists of history, serologic, skin and provocation tests. *Methods:* In years 2007-2008 606 patients (76% women, aged 14-85, average age 42) were referred to our allergology department for further evaluation of penicillin allergy. Diagnostic procedure started with specific IgE measurement, followed by skin prick and intradermal tests with PPL, MDM and suspected antibiotic. If all tests were negative oral challenge was performed. If more than 3 years passed by from the reaction and if the reaction was not severe life threatening direct oral provocation followed serological tests. *Results:* In 35 (5.7%) patients high level of specific IgE to penicillins was confirmed. 274 (45%) patients underwent skin prick and intradermal tests which were positive in 14 (5%) patients. In 8 patients skin tests were positive with PPL and in 6 with MDM. In 427 (70%) patients oral provocation with suspected drug was performed. In only 19 (4.5%) patients hypersensitivity was confirmed. Drug allergy was confirmed in 68 (11%) of all referred patients (72% women, aged 16-79, average age 44) using serologic, skin or provocation tests. In 70% hypersensitivity reaction was immediate with erythema and urticaria. In 30% reaction was delayed with urticaria and maculopapular rash. *Conclusion:* Hypersensitivity to penicillins was confirmed only in minority of all referred patients. Most of them underwent provocation

test, which is time consuming and could be dangerous. In vitro test with high negative predictive value would be useful.

Cross-reactive hypersensitivity to Nonsteroidal Antiinflammatory Drugs (NSAIDs) in children: a case series

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Poster

Background: NSAIDs, mainly ibuprofen, are used extensively among children as analgesics and antipyretics agents. We attempted to characterize the clinical and epidemiological profile of NSAIDs reaction in children. *Material and Methods:* We performed a retrospective analysis of a case series in our Pediatric Allergy Department. The diagnosis of cross-reactive NSAIDs hypersensitivity was made with an oral challenge test. Atopy was evaluated clinically and tested with a standard panel of skin-prick test. *Results:* From January 2000 to December 2008, 14 patients were diagnosed as having cross-reactive hypersensitivity to NSAIDs by oral challenge test. From these patients, 11 (79%) were male patients and 3 (21%) female patients, with a mean age of 9,9 years (range: 5,9-14 years). The oral challenge test was performed with ibuprofen in 3 patients and with aspirin in 12 patients. All the patients (100%) developed facial angioedema (specifically periorbital edema) with the oral challenge test, and 21% generalized urticaria. Respiratory symptoms (dyspnea and/or wheezing and/or cough) were documented in 21% of patients. The average reaction time was 82 minutes (range: 15 minutes to 4 hours). All the patients tolerated acetaminophen. Atopy was present in 86% of patients. A family history consistent with NSAIDs hypersensitivity was documented in 7.1% of patients. *Conclusions:* The cutaneous manifestations, specifically periorbital angioedema, are the clinical key features in the cross-reactive NSAIDs hypersensitivity reactions in these pediatric patients, with positive oral challenge test confirmation. The 86% of our patients were atopic, confirming atopy as a risk factor for the development of NSAIDs hypersensitivity. Acetaminophen is a therapeutic option in the majority of our pediatric patients.

Epidemiology of hypersensitivity drug reactions

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Poster

Introduction: In one poor region such as Republic of Kosovo, which is not only one region in the world (poor of immunological laboratory) working as allergologist-immunologist is very hard and with a very high responsitivity, because you must work in poor conditions that exists and that's are offered to you. Today in a time of e-comunications theoretically every one of us we are trying hard to follow all new knowledges in allergology-immunology including and hypersensitivity to drug reactions. *Materials and methods:* Our work is based on good relation doctor-patient, which means: taking with an accuracy patient anamnesis, after completed with external clinical examinations(inspection, palpitation, auscultation),added completed routine laboratory examinations: blood parameters, renal, liver, rheumatic, urinary parameters, and parasitic investigations. We are lack of one Immunological satisfated laboratory, which means that in our UCCK in Prishtina (University Clinical Center of Kosova) we can proffit not permanently only of detection of total Ig-E and CH50 and it's parameters. Some private laboratory have began to offer determination of specific Ig-E on limited and very poor number of specific Ig analysis. *Results:* Approximately in one city as Gjakova where I work locuited of 105.000 habitants in comparation with capital of Kosova, Prishtina with 600.000 habitants, every day income allergic patients are approximately 10 patients/day presented in allergology-immunology department, and minimum five times more are patients which are presented in other departments: emergency, dermatology, internal department, ENT, pediatric dept. etc. In collaboration with colleagues from UCCK the number is increased for five times it means approximately 50-60 patients/day presented to allergologist-immunologist and 4-5 times more number of patients presented in other departments such as in city Gjakova. Epidemiologically approximately after our determinations minimum 1 patient/day have hypersensitivity drug reaction, it means 0,0003% of our population manifests drug allergy monthly ,and approximately 0.0012- 0.0015% of our population are hypersensitv drug outcome patients which are represented in other departments monthly. The percentage of hypersensitiv patients is higher but some patients negligence their allergic symptoms. But the responsibility belongs also to physicians and Ministry of Health. During 2009 at the Department of Pharmagovigilance on Kosova Medical Agency were reported only 11 drug adverse effect reports (one of them hypersensitivity) from Kosovar physicians while pharmaceutical companies for their products that are registered in Kosova reported 7362 drug adverse effect reports from other states that they products were registred. The frequent drug hypersensitivity is find in; beta-lactams antibiotics (0.000009%, 1/3 of patients), NSAID, beta-blockers, insulin, analgetics, anaesthetics, contraceptives, vitamins, fitotherapy such as propolis, etc. *Conclusions:* We thanks science for e-comunication but our hope is and we to offer to our patients better treatment of allergic deseas. Our goal is to educate our patients to avoid threatening reagens in this case all susceptible drugs which we can suspect after one accuracy completed anamnesis with history of allergic deseas. We can offer "per os"drug provocations test, prick and intradermal increasing test provocations but all the time with our responsibility to not provoke life threatening reactions such as imediated type I Ig-E reactions(Anaphylactic shock ,urticaria and angioedema). In our experience we meat patients and with delayed allergic reactions, serum sickness, vasculitic drug reactions, contact drug dermatitis, photodermatitis after drug intake etc. Every one of us allergologists we very often represent different themes on medias such as a tv and radio to realize one much more patient information and education about allergies in general and exceptionally in drugs and the new approaches of treatment. We hope in future collaboration between us allergologists-immunologists and maybe in one Pharmagovigilance Institute in Kosovo in future which can be not only regional Institute but open to offer services and to neighbors countries: Albania and countries from ex Yugoslavia.

A DRESS epidemic: what was its cause?

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Poster

Background: The association between reactivation of herpes virus (HV) and the drug reaction With eosinophilia and systemic symptoms (DRESS) is currently admitted. We report on 6 cases of DRESS which occurred in 1 month with viral reactivation for which we looked for a common denominator. *Patients and methods:* Three men and three women aged 30 to 69 years were hospitalized between 22/03/09 and 24/04/09. During the following months only 1 case of DRESS per three months was observed. All had taken drugs potentially responsible for inducing DRESS from different classes, 3 to 6 weeks prior to the onset of symptoms and developed DRESS, which all could be classified as class 4 or 5 according to Kardaun et al. criteria. None of the patients was immuno-compromised. The study of HV group activations was done by PCR (EBV, CMV, HHV-6 and 7).both with other viral serologies. *Results:* A primary EBV infection was found in 1 case, associated to HHV-6 reactivation. In 3 cases, EBV and HHV-7 were reactivated. Finally, only CMV reactivation was noted in the 5th case and the 6th case was an isolated reactivation of HHV-7. The serological status for Mycoplasma pneumoniae, Chlamydia pneumoniae, rickettsia, Coxsackie, Parvovirus B19, HIV, HBV, HCV, HV simplex, syphilis were negative or reflected an ancient infection. During the 3 months following thez onset of the DRESS a subsequent infection by HV simplex was observed in 2 cases. *Discussion:* The common denominator in these 6 DRESS is not one particular drug class. Some medicines cause hypogammaglobulinemia with reduced circulating B cells (CD19), production of drug specific LTCD8 and reactive metabolites. The DRESS is not explained solely by cellular response against drug antigens. The pathophysiology of DRESS could be 1.Medication taken 2. Immune response 3. Reactivation (or primary infection) of HV on this immunological drug induced predisposing context, HHV6 but also other HV 4. Stopping medication which restores antiviral immunity 5. Damage cell linked to the intense antiviral CD8 T cell response could explain symptoms and prolonged recurrences and participate to a co-drug sensitization. Some drugs (valproate sodium, amoxicillin) would further entail increasing HHV-6 in vitro replication. We did not identified a single type of HV involved in the reported DRESS epidemic. *Conclusion:* Our 6 cases of DRESS in the short period of one month looks like a viral epidemic. This raises the question of a possible common environmental phenomenon, infectious or not, promoting reactivation of HV and the occurrence of DRESS in context of drug intake.

Steven-Johnson syndrome and toxic epidermal necrolysis. Ten years retrospective analysis

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Poster

Background: Steven-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are very rare but life-threatning conditions, characterized by cutaneous and mucocutaneous involvement. They may represent a different spectrum of a same disease process. Drugs, infections and malignancies are common suspected etiological factors. The aim of this study was to describe the hospitalized cases of SJS/TEN in a central hospital, in a 10 years period. *Methods:* All episodes of SJS/TEN occurred from 1999 to September 2009 were searched in the hospital data base using SJS, TEN and EM ICD-9-CM codes. We reviewed the medical records and evaluated several parameters, namely age, gender, signs and symptoms, affected body surface and possible causes of the episode. *Results:* From the 131 medical records evaluated, in only 31 patients (18 female) was recognized SJS/TEN, with 35.94±27.84 years of mean age at onset of the clinic. 35.5% of sample were aged <12 years old. Only one patient had past history of SJS. Infection was found 6.45±8.10 days before onset of clinic, in 32% of the sample. Respiratory symptoms were present in 29% of sample (respiratory failure in 16%), gastro-intestinal in 10%, target lesions in 16% and erosive lesions in 87%. 90% of the patients had a suspicious implicated drug (42% antibiotics, NSAID's 19%, anticonvulsants 13%, allopurinol 7% and others 6%). The mean time between beginning treatment with the suspicious drug and onset of clinic was 15.89±29.43 days. 10% of sample needed intensive care. EV immunoglobulins were administered in 29%. SJS was found in 15, TEN in 7 and overlap syndrome (OS) in 9 patients. The final suspected causative agents at discharge were drugs in 20, infection and drugs in 8, only infection in 2 and idiopathic in 1 patient. Antibiotics were more likely to cause SJS (OR: 4.5) and to induce clinic in children (OR: 4.1). Allopurinol was related to a worse evolution (p=0.003). Paediatric patients were related to a better resolution (p=0.043). 2 patients died (all TEN, p=0.013) and 4 were transferred to a burn unit (2 SJS and 2 TEN). *Discussion:* The incidence of SJS was 1.7, TEN 0.8 and OS 1.0 cases per million population per year, similar to other studies. Paediatric patients were one third of sample and were related to a better evolution. The most probable causes are common drugs, namely antibiotics and NSAID's. These syndromes can represent a true emergency, with important mortality, especially when the clinical presentation is TEN.

Genetics

Genetic factors of Stevens-Johnson Syndrome with ocular complications in Japanese

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Poster

Purpose: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are acute severe blistering diseases of the skin and are also two of the most devastating ocular surface diseases leading to corneal damage and loss of vision. Although the pathobiological mechanisms underlying the onset of SJS/TEN have not been fully elucidated, the extreme rarity of cutaneous and ocular surface reactions to drug therapies suggests an individual susceptibility. We pre-

viously reported that in Japanese, HLA-A*0206 is strongly associated with SJS/TEN with ocular complications. This clearly suggests that not only environmental but also genetic factors contribute to the etiology of SJS/TEN. In this study, we examined the genetic factors of SJS/TEN with ocular complications. *Methods:* Single nucleotide polymorphism (SNP) association analysis using the candidate gene approach was performed in 80-100 Japanese SJS/TEN patients and 160 Japanese healthy volunteers. For this analysis, candidate genes associated with innate immunity (TLR2, TLR3, MAIL, IL1α), apoptosis (FasL), or allergy (IL4R, IL13, IL4) were selected. The diagnosis of SJS/TEN was based on a confirmed history of the acute onset of high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least 2 mucosal sites, including the ocular surface. *Results:* In three TLR2 SNPs (rs.3840100, rs.3840099, rs.3840097) and five IL1α SNPs (rs.1609682, rs.1894399, rs.2071373, rs.2071375, rs.2071376) that we examined, no association with SJS/TEN was found. Among seven TLR3 SNPs (rs.3775290, rs.3775291, rs.3775292, rs.3775293, rs.3775294, rs.3775295, rs.3775296) that we examined, rs.3775290 and rs.3775296 showed a significant association (p=0.01 and 0.00007, respectively). Among seven MAIL SNPs (rs.2305991, rs.622122, rs.14134, rs.3217713, rs.595788, rs.677011, rs.3821727), rs.595788G/A showed a weak inverse association (p=0.04). Among three IL4R SNPs (rs.1805010, rs.1801275, rs.1805015), Gln551Arg (rs.1801275) showed a significant strong association (p=0.0005). Among four FasL SNPs (rs.929087, rs.2639614, rs.2859247, rs.3830150), rs.3830150 showed a significant association (p=0.002). Among two IL13 SNPs (rs.1800925, rs.20541), Gln110Arg (rs.20541) showed a weak association (p=0.04). SNP -590C/T IL4 (rs.2243250) also showed a weak association (p=0.04). *Conclusion:* Not only environmental factors but also genetic factors may play a role in an integrated etiology of SJS/TEN with ocular complications.

Association of the nonsynonymous histamine N-methyltransferase (HNMT) polymorphism (rs1801105 Thr105Ile) with intolerance to non-steroidal anti-inflammatory drugs

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Poster

Genetic polymorphisms for histamine-metabolizing enzymes are responsible for interindividual variation in histamine metabolism. Two enzymes are known to participate in the degradation of histamine: histamine N-metyltransferase (HNMT, E.C. 2.1.1.8) and diamine oxidase (DAO; ABP1, E.C.1.4.3.6). The HNMT gene is located in chromosome 2q22.1. The most common nonsynonymous HNMT SNP (rs1801105, C568T; Thr105Ile) is associated with reduced thermal stability of the enzyme and with decreased HNMT activity. Aiming to elucidate whether a decrease in histamine metabolism associated to the Thr105Ile polymorphism is related to the development of intolerance to non-steroidal anti-inflammatory drugs (NSAIDs), we analyzed the presence of such polymorphism in genomic DNA from 757 individuals. Among these, 300 of whom had cross-intolerance to NSAIDs, 67 had selective intolerance to NSAIDs and 390 were healthy subjects who tolerated NSAIDs. Overall findings indicate that the frequencies for the 568C allele are higher among patients with intolerance than among healthy controls. The odds ratio, (95% C.I.) were as follows: Overall intolerance patients: 1.43 (1.00-2.07) p=0.053; Cross-intolerance: 1.29 (0.88-1.88) p=0.189; Selective intolerance: 2.34 (1.02-5.35) p=0.045. The influence of the 568C allele is higher in intolerant patients who had not atopy OR=1.67 (1.05-2.64) p=0.031, in patients who had not urticaria OR=1.48 (1.00-2.20) p=0.050, and in patients who had negative prick tests OR=4.14 (1.31-12.99) p=0.013. The average IgE levels were significantly higher in patients with the 568T allele (p=0.022). This study indicates that the HNMT 568C allele moderately increases the risk to develop NSAID intolerance, especially in patients with no atopy, no urticaria and negative prick tests. Although this observation is in contrast with what it could be expected because the 568C allele is related to high HNMT activity, these findings should be interpreted within the context of genetic variability for all histamine metabolising enzymes, and these studies are ongoing.

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HLA-B*35 is associated with nevirapine hypersensitivity in the contemporary Western Australian HIV Cohort study

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Communication

Background: Treatment with the antiretroviral nevirapine is complicated by a hypersensitivity syndrome (NVP HSR) in approximately 5% of those starting the drug. Previous studies have suggested associations with both Class I and Class II major histocompatibility complex alleles suggesting that the immunopathogenesis and pharmacogenetics of this reaction may vary. Prior to 2005 we had described an association between nevirapine rash associated hepatitis and the HLA class II allele DRB1*0101 in predominantly Caucasian men with CD4+%>25% and more recently a Thai group has described an association between HLA-B*3505 and nevirapine rash and HSR. *Material and Methods:* Patients in the Western Australian Cohort study who had received nevirapine and were tolerant for at least 3 months or who had developed NVP HSR were identified. NVP HSR was defined as a minimum of fever and at least one of hepatitis and rash. Co-variates examined included CD4+ count and viral load at commencement of nevirapine treatment, age, gender, viral load, hepatitis C status, and HLA Class I and II alleles. Nevirapine ELISpot to the parent drug and its 12-OH metabolite was performed on consenting patients when stored PBMCs were available. *Results:* Of 401 patients exposed to NVP between March 1993 and November 2009, 15 (3.7%) were identified who met criteria for NVP HSR. 10/15 (67%) patients were White and 5/15 (33%) Asian origin. 8/15 (53%) of patients had a combination of both fever and eosinophilia and at least one of hepatitis and rash. Factors significantly associated with NVP HSR included HLA-B*35 [OR 4.4, p=0.016]. There was no association between NVP HSR and CD4+ count at treatment although in patients exposed to nevirapine mean

CD4+ count at treatment was higher > 2005 versus < 2004 (517 + 308 vs. 433 + 277 p=0.014). Of the 6/15 patients with cells available for nevirapine ELISpot, 2 patients (33%) were positive with both sharing the haplotype HLA-A*2407-B*35-C*0401. *Conclusions:* The changing epidemiology and ethnicity of our population with more recent cases of NVP HSR occurring in those of Southeast Asian and Indonesian background is a potential explanation for the association between HLA-B*35 and NVP HSR and the current lack of association between pre-treatment CD4+ count and NVP HSR in our cohort.

Cysteinyl leukotriene receptor 1 polymorphisms in Portuguese patients with nasal polyps and asthma

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Poster

Background: The relation of different asthma phenotypes with the cysteinyl leukotriene receptor 1 (CYSLTR1) nucleotide polymorphisms (SNPs) has been investigated for years. However, the results are inconsistent, which may be partially explained by the geographically different populations studied. *Objective:* To study CYSLTR1 polymorphisms in Portuguese patients with nasal polyposis and asthma, with and without NSAIDs hypersensitivity. Patients and methods: Twenty six patients with NP and asthma from our drug allergy consultation were randomly selected, 13 also with NSAIDs hypersensitivity (9 F/4 M, mean age 57.5+10 years) and 13 without (9 F/4 M, mean age 54+10 years). CYSLTR1 was analysed by direct sequencing after PCR amplification. The CYSLTR1 gene was screened for SNPs and we then investigated their eventual associations with asthma phenotypes. Twenty four healthy adults were used as controls. *Results:* Three SNPs were identified, the already known 927 T>C, 899 G>A, as well as an SNP not described in the literature – 764 T>A, which results in the substitution of the coded aminoacid. The 927 C allele was slightly more frequent in the NSAIDs hypersensitivity patients (23,1% - 3/13) than in NSAIDs tolerant patients (16,5% - 1/13 in the nasal polyps and asthma group and 4/24 in the control group). None of the individuals studied presented the 899 G>A SNP. Regarding 764 T>A, a higher frequency was encountered in the nasal polyps and asthma without NSAID hypersensitivity group (58.3% - 7/12) when compared with the NSAID hypersensitivity group (21,3% - 3/13) and controls (33.3% - 8/24). There were no gender differences in any of the studied SNPs. *Conclusion:* The 764 T>A, a previously undescribed SNP was associated in our patients with a higher risk of nasal polyps and asthma without NSAIDs hypersensitivity. However, the control group also had a relatively high incidence of this allele. Previous studies associating the 927 T>C SNP with asthma diagnosis in males and 899 G>A SNP with asthma in females was not confirmed. Further studies in the same population with a larger number of individuals are therefore needed to determine if the 764 T>A SNP is relevant in this subgroup of patients.

Urticaria/angioedema induced by cross-intolerance to NSAIDs: a pharmacogenetic study

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Poster

Background: NSAIDs are actually the compounds most frequently involved in hypersensitivity drug reactions. There are at least three different mechanisms: IgE-mediated, and T cell-mediated (immunological specific mechanisms) and cross- intolerance (CI) (immunological non-specific). The latter is the most frequent, being the skin the main organ affected. However, airway involvement has been the best studied model. The association between CI and different single nucleotide polymorphisms in arachidonic acid pathway-related genes has been described in patients with respiratory symptoms or chronic urticaria, but up today the number of cases is rather limited and selected populations are heterogeneous. In this preliminary study, we analysed the association between CI to NSAIDs and several SNPs in a group of genes involved in the arachidonic acid metabolism pathway in patients with acute urticaria/angioedema. *Methods:* The population was obtained from several Allergy Services integrated in the Spanish network RIRAAF. Cases included had to have at least two episodes of cutaneous symptoms after the intake of two or more NSAIDs from different chemical groups. We studied several SNPs in ALOX5AP, ALOX5, PTGS1, PTGS2, and LTC4S genes by real-time PCR, and melting curves analysis. *Results:* From a total of 445 hypersensitivity patients with CI to NSAIDs, 300 presented urticaria/angioedema. We also included a similar number of age- and sex-matched healthy controls. No differences between the two groups for any of the SNPs analyzed were found. When compared with data from NCBI, we have found that our genotypic and allelic frequencies are similar. *Conclusions:* Urticaria/angioedema was the most relevant clinical symptom in patients with CI to NSAIDs. A significant association between the SNPs studied and CI was not found. Further studies are required to analyze the role of prostaglandins and leukotrienes in the etiopathogenesis of cutaneous manifestations in subjects with CI to NSAIDs.

Immunology - Immunotoxicology

Investigation immunomodulatory effect of anticoagulant warfarin by experimental caused contact hypersensitivity reactio

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Poster

Introduction: Depending on the chemical agent and dose, toxicity for immune system is manifested as skin contact hypersensitivity (CH), inflammation or immunosuppression. *Objectives:* The objectiv was to examine the local immunodermatotoxicy first generation anticoagulant warfarin, which is aplicate on the skin. *Method:* The analysis of the effect of warfarin is used contact hypersensitivity

(CH) reaction to the hapten dinitrochlorobensen (DNCB) in rats the Dark August stock. Statistical data is made use of descriptive statistics for the calculation of average values and standard deviations for in vivo (ear swelling assay), a mean value and error for the in vitro tests (MTT test for T cell proliferation) and using the Student t-test. *Results:* Local epicutane application warfarin before induction reaction CH to DNCB leads to a decrease that intensity. It is accompanied by changes of draining lymph nodes in the skin caused by this agent. Noticeable changes in the lymphn part may be a consequence of changes in the system level caused proliferativni activities mononuclear cells, as well as by the presence of warfarin in the circulation. By animals that is not later induced CH to DNCB also leads to functional changes in cells draining lymph nodes. Application of warfarin before the phase expressions CH leads to a reduction costimulation activities ephiderm cells, but does not affect the change in ICAM-1 expression. Conclusion: Changes in functional activities of cells draining lymph nodes after local epicutane applications warfarin in the induction phase of CH, as well as before the phase expressions illustrate immunotoxicy local effect of warfarin.

Validation of an oral exposure mouse model for the prediction of drug hypersensitivity reactions using a reporter antigen

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Poster

Adverse drug reactions (ADRs) are the main cause of black box warnings or even drug withdrawals. Due to their idiosyncratic (and therefore rare) nature, ADRs are often not noticed until a drug has been marketed and used in the general population for some time. So far there are no validated in vitro or in vivo models to predict possible ADRs and there is a demand for pre-clinical screening tools. Previous studies have shown the possible predictive value of an oral mouse model using the Reporter Antigen (RA), Trinitrophenyl-Ovalbumin (TNP-OVA). Using this model, the sensitizing capacity of e.g. Diclofenac (DF) and D-Penicillamine (D-Pen) have been demonstrated. In the current study we extended this data by testing the analgesic drug acetaminophen (APAP) and its non hepatotoxic regioisomer (AMAP), the antibiotic Ofloxacin (OFLX), and the anti-diabetic drug Metformin (MET) in the oral model using TNP-OVA as read out. We evaluated whether these compounds were able to cause sensitization via oral exposure. Therefore, C3H/HeOuJ mice were dosed by oral gavage (30/100/300 mg/kg bodyweight for APAP and AMAP, 100/300/1000 mg/kg for OFLX and 50/100/500 mg/kg for MET) for 7 consecutive days. At the first day of exposure the mice received an intraperitoneal injection of TNP-OVA. After 15 days they were ear-challenged with TNP-OVA and Delayed Type Hypersensitivity (DHT) was assessed 24 hours later. One week after challenge, the draining lymph node was removed and TNP-specific antibody secreting cells (ASC) were determined. Serum collected on day 21 was analyzed for TNP-specific antibodies. Both APAP and OFLX showed a significant dose dependent increase in DTH responses to ear-injection with TNP-OVA, whereas both AMAP and MET did not. ASC and serum antibodies against the RA were only detected in some individual drug treated mice. In conclusion, the present oral exposure model has the ability to identify drugs known to cause adverse immune reactions, with the increase of a DTH response as most important parameter.

Microbial compounds alter drug induced immune responses

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Communication

Many drugs are known to induce adverse immune reactions in susceptible individuals and may result in clinical diseases. Etiology of these adverse immune reactions is not completely understood, but known risk factors are e.g. genetic background, age, and metabolism. Furthermore, microbial status of the patient has been implicated in the induction of drug allergies. Especially viral infections have been associated with the initiation of these hypersensitivity reactions. The liver is a key organ in the metabolism of orally taken drugs, generating active metabolites and possibly haptens of the original compound. Furthermore, the liver is an immunological active organ with a distinct lymphocyte population responsible for tolerance induction to orally administered antigens. Viral or bacterial infections in the liver could disrupt the homeostasis by inducing liver injury and changes in lymphocyte population, leading to the induction of immunological responses to harmless antigens. In these studies we investigated the effect of LPS and poly(I:C) as model compounds for GRAM-negative bacteria and viruses respectively on acetaminophen (APAP) induced liver injury and liver lymphocyte population. C57Bl/6 mice received via oral gavage a single dose of 300 mg/kg APAP and 24 hours later liver enzymes were assessed, liver lymphocytes were isolated and analyzed, and liver histology was evaluated. The administration of APAP elevated the plasma levels of ALT and AST. The non-hepatotoxic regioisomer AMAP did not induce liver injury at this dose. Furthermore, APAP treated mice had increased numbers of liver lymphocytes. Especially the amount of neutrophils was elevated. When mice received a single dose of LPS intraperitonealy 6 hours after APAP administration, APAP-induced liver injury was increased. Strikingly, poly(I:C) injection 6 hours after APAP reduced both APAP-induced liver injury and changes in liver lymphocyte population. In conclusion, APAP induced liver injury is attenuated by poly(I:C) and increased by LPS which might affect systemic responses to APAP.

Anti-cetuximab IgE ELISA set-up for detection of patient at high risk of cetuximab-induced anaphylaxis

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Poster

Background: Cetuximab is a chimeric mouse-human IgG1 monoclonal antibody against Epidermal Growth Factor Receptor. This therapy is approved for use in colorectal cancer and squamous-cell

carcinoma of the head and neck. A high prevalence of hypersensitivity reactions (HSR) to cetuximab after the first infusion has been observed in some areas in the world. The aim of this study was to evaluate the potential an anti-cetuximab IgE enzyme-linked immunosorbent assay (ELISA) in the analysis of the reactions observed among our patients. *Methods:* An ELISA detecting anti-cetuximab IgE has been setup and performed on 92 patients treated with cetuximab in François Baclesse Center, Caen, France. Two control groups of healthy blood donors (Etablissement Français du Sang, Caen and Rouen, France) were also analysed. *Results:* 21/213 (9.9%) patients treated had hypersensitivity reactions (HSR) after the first infusion with 2 deaths. Anti-cetuximab IgE were detected in 24/92 (26.1%) of the patient tested versus 17/58 (29.3%) in healthy blood donors from Caen and 16/59 (27.1%) from Rouen (p=ns). HSR was observed in 14 out of these 92 patients (15.2%), 8/14 (57.1%) being quoted as grade 3-5. Anti-cetuximab IgE were found in 7/8 (87.5%) of these grade 3-5 patients as compared to 17.9% in patients with no HSR (p=0.0002). Predictive value of anti-cetuximab IgE for hypersensitivity events was calculated using Receiving Operating Characteristic (ROC) analysis. Using a threshold of 30 arbitrary units of IgE (AUE) anti-cetuximab detection by ELISA showed a sensitivity of 87.5%, a specificity of 79.8%, a positive predictive value of 33.3% and a negative predictive value of 98.5% when considering HSR grade 3-5. *Conclusion:* Anti-cetuximab IgE ELISA detection could represent a valuable tool to help the physician to anticipate an anaphylaxis episode following first cetuximab infusion and select an alternative treatment when available.

Development of a neutrophil cell culture system to define the sensitizing potential of chemical allergens that acquire protein reactivity through metabolism

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Poster

Background: Legislation prohibiting animal testing for evaluating the sensitizing potential of cosmetics has resulted in the search for alternative in vitro approaches. Several assays based on monitoring dendritic cell maturation have been shown to be useful at predicting the sensitizing potential of directly protein-reactive chemicals. However, pro-haptens", which gain reactivity though metabolic activation, frequently yield negative results. The aim of this study was to compare metabolic activity in the monocytic cell line THP-1 and neutrophils and (2) explore whether measurement of neutrophil IL-8 secretion might be a useful assay for the prediction of the sensitizing potential of pro-haptens. *Methods:* Enzyme expression was measured by immunoblotting with specific antibodies. Furthermore, neutrophils and THP-1 cells were cultured either together or in isolation with the model hapten 1,4 dinitrochlorobenzene (DNCB) and the pro-hapten eugenol for 24 h, prior to the analysis of IL-8 secretion by ELISA. *Results:* CYP1A1, 1B1 and 3A4 expression was not detectable in THP-1 cells or neutrophils (limits of detection using recombinant CYPs; 1A1, 1fmol, 1B1 10 fmol, 3A4 4 fmol). Myeloperoxidase was detected in neutrophils (from 0.75 μg/ml cell protein), but not THP-1 cells (up to 40 μg/ml protein). Significant quantities of IL-8 was measured in culture supernatant when DNCB was cultured with THP-1 cells (control, 0.15±0.05 ng/ml; DNCB, 2.72±1.46 ng/ml) and neutrophils (control, 0.01±0.01 ng/ml; DNCB, 1.56±0.96 ng/ml) in isolation. In view of the fact that IL-8 secretion was not enhanced when DNCB was cultured with both cell types (control, 0.09±0.05 ng/ml; DNCB, 1.86±0.14 ng/ml), all subsequent experiments comparing IL-8 secretion from THP-1 cells and neutrophils, were performed with the cells cultured separately. Eugenol treatment (10-500 μM) of THP-1 cells was associated with the secretion of low levels of IL-8 (control, 0.15±0.05 ng/ml; eugenol 250 μM, 0.36±0.04 ng/ml). In contrast, IL-8 secretion from eugenol-treated neutrophils was significantly higher (control, 0.01±0.01 ng/ml; eugenol 250 μM, 1.30±0.60 ng/ml: $P < 0.05$). *Conclusion:* These data suggest that the development of a system using neutrophils may be useful for the prediction of the sensitizing potential of chemicals that gain protein-reactivity through metabolism. This work was conducted in the MRC CDSS [grant number G0700654]. The project received funding from EU Framework 6 project Sens-it-iv.

Th17 cytokines in penicillamine-induced autoimmunity in Brown Norway rats

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Poster

Introduction: At present, idiosyncratic drug reactions (IDRs) are unpredictable largely due to a lack of mechanistic understanding; however, their clinical characteristics suggest that they are immune-mediated. Penicillamine-induced autoimmunity in Brown Norway (BN) rats has been utilized as an animal model for mechanistic studies of one type of IDR because it mimics the autoimmune syndromes that this drug causes in humans. It has been shown that Th17 cells play a central role in many types of autoimmune diseases. This study was designed to test whether Th17 cells are involved in the pathogenesis of penicillamine-induced autoimmunity and to establish an overall serum cytokine/chemokine profile for this IDR as a possible template for other types of IDRs. *Methods and Results:* BN rats were treated with D-penicillamine (150 mg/kg in drinking water). In animals that developed autoimmunity, IL-6 and TGF-1, known to be driving forces of Th17 differentiation, were increased both during the first week and also a few days before the onset of autoimmunity; however, no significant changes in these cytokines were observed in animals that did not develop autoimmunity. IL-17, one of the characteristic cytokines produced by Th17 cells, was increased in sick animals. In addition, following the initial increase of IL-6, the serum concentration of IL-22, another characteristic cytokine produced by Th17 cells, was found to be elevated early (3 days after treatment) and when the rats developed clinical evidence of autoimmunity, which suggests that Th17 cells are involved in this autoimmune syndrome. However, in ongoing studies to determine the source of IL-17, it appears that other cells such as macrophages and/or NK cells may be a major source of IL-17. In total, 24 serum cytokines/chemokines were determined by a Luminex assay and revealed a dynamic process. For example, a peak in IL-13 at an early

time point predicted which animals would develop autoimmunity. *Conclusion:* This study demonstrates that Th17 cytokines are involved in penicillamine-induced autoimmune disease in BN rats. However, more research is needed to elucidate the involvement and mechanism of Th17 cells in this autoimmunity. Some cytokine changes that predicted which animals would develop autoimmunity occurred early and this could lead to methods to predict who would develop other IDRs. These studies were supported by grants from CIHR.

An investigation of the role of b cells in nevirapine-induced skin rash

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Poster

Introduction: Nevirapine (NVP) is used to treat AIDS but it is associated with a high incidence of idiosyncratic drug reactions (IDRs). We found that NVP also causes a skin rash in Brown Norway (BN) rats with characteristics very similar to the rash in humans. NVP-induced skin rash in BN rats is clearly immune-mediated but the details of how this immune reaction is initiated are unknown. We have shown that depletion of CD4 T cells is protective but depletion of CD8 T cells is not. An increase of number of B cells and % of B cells expressing MHC II after NVP treatment, as well as a spike in IgE on day 7 of NVP treatment suggested the possible involvement of B cells in the pathogenesis of this IDR. Rituximab has been used clinically to deplete B cells for the treatment of several immune-mediated diseases such as multiple sclerosis. In this study, we used an anti-mouse CD20 antibody obtained from Genentech to deplete B cells to see if this would prevent the rash. *Methods and Results:* 28 Female BN rats were divided into seven groups. Each group received either an anti-CD20 injection (i.v., 1 mg/rat) or vehicle control at different times before or during NVP treatment (150 mg/kg/day in food for 21 days). A single dose of antibody depleted B cells in the blood by about 90% in 3 days. Two weeks after the injection, B cell levels were still reduced by about 80% in blood, 15% in auricular lymph nodes and 10% in the spleen. A less severe rash was observed in all animals that received antibody injections compared to the NVP alone group. A group of animals receiving 2 antibody injections on days 4 and 7 following initiation of NVP treatment had a 5 day delay of onset of disease. Because of the large number of B cells remaining in the spleen, we performed a splenectomy on a group of animals and repeated the experiments but the results were the same. *Conclusions:* Depleting B cells using the anti-mouse CD20 antibody did not change the incidence of NVP-induced rash which suggests that they are not essential. However, it did appear to decrease the severity and delay the onset of rash, and total depletion was not achieved. Alternative ways to test the involvement of B cells will be required. With these and related studies we will better understand the mechanism of NVP-induced skin rash and, in turn, this may provide possible ways to prevent and/or treat IDRs. This work was supported by grants from CIHR.

Species differences in the interaction between LPS and paracetamol, diclofenac and ketoconazole in precision-cut liver slices

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Poster

Idiosyncratic drug reactions (IDRs) are adverse drug reactions that occur without obvious relation to time or dose and are unpredictable and sporadic. IDRs may arise due to inflammatory episodes concomitant with exposure to the drug that renders the liver more sensitive to injury resulting in increased toxicity. The aim of the study is to investigate the influence of an inflammatory reaction on the toxicity of drugs and species differences therein using human and animal precision-cut liver slices (PCLS). The technology of PCLS is receiving increased attention as a potential in vitro toxicological model because PCLS retain the normal tissue architecture of an intact liver where all cell types are present in their natural environment. Mouse and human PCLS were incubated with paracetamol (APAP), diclofenac (DF) or ketoconazole (KCZ) alone or in the presence of lipopolysaccharide (LPS) to induce an inflammatory reaction. Cell viability (ATP, liver enzyme leakage) and cytokine production were assessed. Both APAP and DF, but not KCZ, were more toxic in mouse than human. LPS had no influence on APAP and DF toxicity in mouse or human when assessed by viability tests. However, APAP and DF decreased the LPS-induced IL-1β production in both species while IL-6 production was only decreased in mouse PCLS. APAP increased LPS-induced TNF-α production in mouse but strongly reduced it in human PCLS, while in both species TNF-α production was increased by DF alone, but not by APAP alone. In contrast to APAP and DF, KCZ toxicity was increased in the presence of a non-toxic dose of LPS in mouse PCLS, while KCZ did not decrease the LPS-induced IL-1β production. In conclusion, KCZ toxicity is aggravated by LPS and clear species differences were identified in the effect of APAP, DF and KCZ on the inflammatory reactions induce by LPS. The role of cytokines in the effect of LPS on the toxicity of KCZ needs to be further investigated. Based on these results PCLS appear promising as an in vitro translational model to unravel the mechanism behind IDRs and to find biomarkers that can detect them.

Skin tests protocol for the prevention of hypersensitivity

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Poster

Background: Oxaliplatin (OHP) is a third generation platinum salt indicated for the treatment of colorectal and other gastrointestinal cancer. Reports of hypersensitivity reactions to oxaliplatin, also very

severe (about 2% of patients), are progressively increasing and can determine the stop of a very effective therapy. The clinical pictures reported are typical of IgE-mediated reactions and occur during the infusion of the chemotherapeutic drug. *Objective:* Aim of the study was to evaluate the ability of skin tests to predict patients at risk to develop hypersensitivity reactions to OHP. *Methods:* The protocol was approved and shared by the European Network on Drug Allergy (ENDA), the interest group on Drug Allergy of the European Academy of Allergy and Clinical Immunology. Skin tests were performed in patients in treatment with OHP combinations, 30 minutes before the start of chemotherapy, beginning by the second administration. In the first step, prick test at the concentration of 1 mg/ml was administered. In case of negativity, patients received an intradermal test at the concentration of 0,1 mg/ml of oxaliplatin. If the test was positive, OHP was administered with a 12-step desensitisation protocol. In this schedule OHP is infused at 3 different concentrations, with an increasing rate of infusion, in about 6 hours. *Results:* At this time, 86 patients, 58 male and 28 female, were evaluated. The median age was 68.5 years (range 47-82); 69 patients had colon disease and 14 patients had biliary tract and pancreas tumor. 37 patients received the chemotherapy in adjuvant setting, 49 for metastatic or non resectable cancer. A total of 694 test was performed (median 8 per patient). Three patients had positive intradermal test, without any previous symptom of hypersensitivity reaction, respectively before the seventh, eleventh and thirteenth infusion of OHP. Two patients, one male and one female, underwent the desensitization protocol, that provoked only mild itchy urticaria. Both the patients ultimated the established schedule of chemotherapy. In the third case we decided to discontinue the therapy, because the woman had yet received 10 administrations of OHP. *Conclusion:* In this ongoing study, preliminary results demonstrate that skin test with OHP are very useful to prevent hypersensitivity reactions to this drug. In this type of patients a 12-step desensitisation protocol permit to continue to administer this very effective platinum salt.

Are pharmacovigilance algorithms trustful for the diagnosis of drug hypersensitivity?

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Poster

Background: Drug hypersensitivity reactions can represent about 20% of all adverse drug reactions (ADR). There are algorithms used to classify ADR and establish a probability, but its usefulness for drug hypersensitivity (DH) reactions has been questioned. The aim of this study was to compare the results of the application of the pharmacovigilance algorithm in DH paediatric patients and compare the results with the drug hypersensitivity workup (DHW). *Material and Methods:* An algorithm for ADR pharmacovigilance (World Health Organization – 1991) used by the Portuguese drug authority was applied in paediatric patients with DH. Classification from algorithm was compared with the results of DHW, including drug provocation test with the culprit drug. *Results:* 44 patients were evaluated (30 male), with a mean age of 9.75±4.37 years. Antibiotics were involved in ADR in 80% and NSAID's in 21%. Cutaneous symptoms were present in 96% of the sample, respiratory in 7%, gastro-intestinal in 11% and anaphylaxis in only 1 patient. Most of the reactions were delayed (86%). The implicated drug was administered for the first time in 50% of sample. 16% had previous reactions with the same drug and 18% had previous reactions with other drugs. The DHW was positive in only 7 patients (16%), however using the algorithm 32 were classified as probable (16% with positive DHW), 9 as certain (22% with positive DHW) and 3 as possible (all with negative DHW). If we considered the "possible" classification as negative and the others as positive, we have a sensitivity of 1, specificity of 0.081, positive predictive value (PPV) of 0.17 and negative predictive value (NPV) of 1. No statistically significant association was found between DHW and the algorithm. *Conclusion:* The algorithm used for the classification of ADR is not reliable for DH. Most of patients with "certain" classification by the algorithm had negative PT and the majority of the positive PT were classified as "probable". The algorithm presented a high sensitivity and NPV but low specificity and PPV. The allergological study with clinical evaluation, in vivo and in vitro tests, but mainly the PT remains necessary for a correct DH diagnostic.

Identification of haptenised peptides from human serum proteins databank using a bioinformatics approach

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Communication

Allergy is a multi-form, multi-organ systemic inflammation reaction, characterized by an immune response to one or more allergens. According to the World Health Organization, allergies are the fourth most prevalent global affliction; with one in three people affected. OMS predict that the prevalence of allergy will be one in two by 2010. Although not common, allergies can be lethal. According to the hapten hypothesis, chemical allergens need to bind to proteins to be recognized by the immune system. The objective of this study is to predict haptenised proteins using a bioinformatic approach; we used B-lactam antibiotics as a model. Using the Uniprot sequences databank, 6020 sequences of human serum proteins were downloaded and transferred in our in-house automatic homology modeller (Modeome);1536 models were constructed and stored in the human serum models databank. We start from the natural target of the beta-lactam antibiotic: the Penicillin Binding Protein (PBP). In previous studies, we analysed the catalytic site and we simulated the bonding reaction between beta-lactam antibiotic and PBP. We used mixed methods (Quantum Mechanic / Molecular Mechanic) to determine the transitional state geometry and nature of amino acids involved in the reaction. The in-house software, Surfing Molecules (SUMO), was used to investigate similar bonding sites on other proteins of the databank. This software describes surface of proteins based on chemical functions of amino acids which are coded in "SuMo objects". These objects are superposed to compare the proteins surface, and a scoring function is applied to rank similar sites. For each bonding site, the presence of lysine or serine amino acid in catalytic position was checked to validate if the beta-lactam mole-

cule could be bonded to the site. After analysis of all potential bonding sites, sequences centred on the catalytic amino acid, were identified. We then predicted the affinity of each peptide for different HLA alleles using a web based software. Several candidate peptides sequences are currently under synthesis and will be bonded to betalactam molecule. These complexes will be tested in vitro for their affinity to HLA peptides. These bioinformatics tools could be extended to other drugs to found haptenised proteins and drug bonding peptide.

Cellular immunology

Possible involvement of CD16 monocyte at the dermo-epidermal junction in the epidermal damage of toxic epidermal necrolysis

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Poster

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are caused by drug administration, and are characterized by epidermal detachment due to keratinocyte apoptosis. The pathogenesis of epidermal apoptosis remains controversial, although roles for cytotoxic lymphocytes, soluble Fas ligand, and granulysin have been proposed. We examined the role of monocytes, which are observed abundantly in the epidermis of skin lesions, and analyzed the properties and functions of these cells in SJS/TEN. Immunostaining of skin sections was performed to examine the membrane markers of monocytes infiltrating into skin lesions. Monocytes infiltrating into skin lesions expressed CD16. These cells were located along the dermo-epidermal junction not only in the involved lesions, but also in very early lesions without epidermal damage, suggesting that infiltration of CD16 monocytes is a cause, rather than a result, of epidermal damage. These observations led us to examine whether a unique accumulation of CD16 monocytes at the dermo-epidermal junction participate in the epidermal damage of SJS/TEN. Compared to CD14 monocytes, CD16 monocytes produce higher levels of proinflammatory cytokines upon stimulation, but they do not show increased IL-10 production. In vitro experiments revealed that CD16 monocytes collected from peripheral blood mononuclear cells of normal subjects showed cytotoxic effects against cultured keratinocytes treated with IFN-gamma. Cytotoxicity was attenuated by 70% with the addition of anti-TNF-alpha antibodies. Excessive TNF-alpha production in CD16 monocytes plays an important role along with IFN-gamma in epidermal damage associated with SJS/TEN. These results indicate that anti-TNF-alpha therapy and the inhibition of monocyte activation will be useful in the treatment of SJS/TEN.

Interaction of Amoxicillin and TLR agonists with dendritic cells from patients with non-immediate allergy to betalactams. Modification of dendritic cell phenotype and lymphocytes response

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Poster

Background: Dendritic cells (DC) are essential antigen-presenting cells and express toll like receptors (TLR) that recognize pathogen-associated-molecular-patterns (microbial and virus products), initiating the maturation process. Drugs as amoxicillin and heparins have showed to induce DC maturation in allergic patients to these drugs, although lower than those induced by TLR agonists. We wanted to analyze whether the combination of amoxicillin with TLR agonists amplify the DC maturation and trigger T cell response in allergic patients. *Methods:* We studied 9 patients with a non-immediate reaction to amoxicillin and 11 tolerant controls. Monocytes-derived-DC were cultured with amoxicillin with/without TLR4, 7, 8 and 9 agonists. For proliferation assays, lymphocytes were cultured with DC at the same combination as above. Studies of the DC maturational status (increased expression of HLA-DR, CD80, CD86 and CD83), lymphocyte transformation test (LTT) and cytokines secretions were performed by flow cytometry. *Results:* In patients, amoxicillin induced a semi-maturation of DC being lower than LPS and resiquimod (TLR4 and 8 agonists respectively) made it. The combination of amoxicillin plus these agonists induced higher maturation than with them alone. Lymphocytes showed a positive proliferation in presence of AX, LPS and resiquimod. Again, when the drug was added with TLR4 or TLR8 agonists the proliferation was higher. The cytokine production in DC after stimulation with amoxicillin, TLR4 or TLR8 agonists showed a Th1 pattern in all of then being lowest in amoxicillin-stimulated-DC. In the LTT, amoxicillin induced a Th0/1 pattern whereas TLR4 or TLR8 agonists induced a strong increase of Th1 cytokines. The combination of amoxicillin with TLR4 or TLR8 agonists induced an increase in the Th1 cytokine production being stronger than those produce by each molecule alone. We did not observe any change with TLR7 or TLR9 agonists. In controls, we didn't observe DC maturation, lymphocyte proliferation and cytokine production with amoxicillin whereas with LPS or resiquimod we observed the same changes as those produced in allergic patients. *Conclusions:* These results show that amoxicillin in combination to TLR4 or TLR8 agonists boost the DC maturation and modulate the specific response towards a Th1 response in patients with non-immediate reaction to this drug.

Differential gene expression in drug hypersensitivity reactions: induction of alarmins in severe bullous diseases

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Poster

Background: Delayed hypersensitivity reactions to drugs can be life-threatening and constitute a growing problem in clinical practice. Al-

though drug-specific T cells seem to be involved, the cellular and molecular bases of their etiopathology are not fully understood. *Objectives:* To study the molecular mechanisms underlying the pathogenesis and the clinical heterogeneity of cutaneous delayed hypersensitivity reactions to drugs. *Methods:* We characterised the gene expression profiles of peripheral blood mononuclear cells (PBMCs) isolated from patients during the acute phase of the reaction and upon resolution of clinical symptoms using a cDNA array technology. Low density arrays were used to confirm differential expression of selected genes during the acute disease in patients and to compare gene expression in patients and exposed control donors by quantitative real-time PCR. *Results:* Eighty-five genes were found to be differentially expressed during the acute phase of cutaneous drug-induced delayed hypersensitivity reactions. Furthermore, ninety-two genes with distinct expression patterns in severe and benign diseases during the acute phase were identified. PBMCs from patients with severe bullous diseases showed a characteristic gene expression pattern with lower expression of genes encoding T cell specific proteins and high expression of cell cycle-related genes and genes coding for inflammatory-related mediators among which several endogenous damage-associated molecular patterns (DAMPs) or alarmins were found. *Conclusion:* Distinct gene expression profiles in PMBCs define benign and severe clinical entities. Over-expression of endogenous DAMPs in Stevens-Johnsons syndrome and toxic epidermal necrolysis.

Expression of α-defensin 1-3 in T cells from severe cutaneous drug-induced hypersensitivity reactions

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Poster

Background: Cytotoxic T cells seem to be the main effector cells in Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). However, recent data support a role of the innate immune system in the etiopathology of drug-induced cutaneous reactions. *Methods:* Gene expression was analyzed by quantitative and end point RT-PCR in PBMCs or selected subpopulations from patients during the acute disease and upon resolution of the clinical symptoms. Flow cytometry and immunohistochemistry were carried out to verify protein expression in mononuclear cells from peripheral blood and skin infiltrates. Peptide concentration was evaluated by ELISA in plasma and blister fluid samples. *Results:* We herein describe DEFA1A3 gene expression in PBMCs from patients with bullous diseases induced by drugs. Protein expression was confirmed in mononuclear cells from the patients, including monocytes, NK cells, CD4+ and CD8+ T cells. Gene expression analysis by RT-PCR unveiled transcription in CD4 and CD8 peripheral blood T cells. Further analysis of protein content by flow cytometry revealed higher protein levels in CD56+CD3+ lymphocytes from SJS/TEN patients. Immunohistological analysis was used to confirm gGene expression and protein content was confirmed in blister fluid of mononuclear cells and in the dermal mononuclear infiltrate by immunohistological analysis. Peptide levels were estimated by ELISA to be 3 to 175-fold higher in blister fluids as compared to simultaneously drawn plasma samples. *Conclusion:* Upregulation of innate immune molecules such as α-defensins 1-3 in T cells from patients with SJS and TEN mayight be involved in the etiopathology of these life-threatening diseases induced by medications.

The cellular source and function of allergen-induced interleukin 2

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Poster

Drug hypersensitivity reactions can be classified as delayed or immediate, with type 1 and type 2 T lymphocyte activation respectively implicated in the mechanism of action. We have demonstrated previously that prolonged topical exposure of BALB/c strain mice to the chemical allergens 2,4-dinitrochlorobenzene (DNCB) or to trimellitic anhydride (TMA) stimulates selective type 1 or type 2 immune responses. Using these haptens as chemical probes to polarize the immune system, we have investigated events occurring during the initiation of allergic responses. We have demonstrated that a single application of allergen results in the expression of discrete cutaneous cytokine profiles and the migration of Langerhans' cells (LC) with different tempos. Thus, DNCB is associated with a rapid and transient increase in cutaneous IL-2 and rapid LC migration whereas treatment with TMA is characterized by early increases in cutaneous IL-10 and more delayed LC migration. Cytokine expression in draining auricular lymph node cells (LNC) was monitored also. DNCB exposure provoked rapid production of IL-2, and, with somewhat delayed kinetics, IL-17 and interferon-gamma (IFN). Relatively low levels of these cytokines were recorded after similar treatment with TMA or vehicle at these early time points (6 to 16 hr). Removal of cells expressing B220, a B cell marker, was without effect on IL-2, IL-17 or IFN expression. Depletion of cells expressing CD25, the high affinity IL-2 receptor expressed primarily by activated T cells and natural killer (NK) cells, reduced IL-2 expression and abrogated secretion of IL-17 and IFN. Removal of cells expressing the NK cell marker CD49b also resulted in reduced production of these three cytokines. Finally, depletion of cells expressing the dendritic cell (DC) marker CD11c inhibited IL-2 expression completely as well as reducing IL-17 and IFN secretion. These data suggest that there are complex interactions between DC, NK and T cells in DNCB-activated LNC that result in the observed cytokine secretion profile and subsequent Th1 polarization of the immune response. DNCB-activated DC are the likely source of the IL-2 that serves to stimulate the production of IL-17 and IFN by other skin draining LNC, probably NK and activated T cells. Furthermore, these observations suggest that it may be possible to discriminate between different types of allergen on the basis of profiles of skin cytokines induced upon first exposure.

T-cell reactivity with abacavir in drug naive individuals

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Poster

Introduction: A potentially life-threatening reaction accompanies the use of HIV nucleoside analogue abacavir (ABC) in approximately

5% of Caucasian individuals. A prospective study involving 1956 patients confirmed a strong association between the carriage of the HLA-B*5701 allele and ABC hypersensitivity in HIV-infected individuals. Since it has been shown that ABC-specific CD8+ T-cells can be found in ABC-naïve, healthy HLA-B*5701 positive individuals, we aimed at identifying the ABC-recognition pattern of ABC-specific T-cells. *Methods:* Peripheral blood mononuclear cells (PBMC) from ABC-naïve, HLA-B*5701+ healthy donors were stimulated for 13 days with ABC. Expansion of ABC-reactive cells was monitored by means of IFNy intracellular staining on FACS analysis after a short in vitro ABC-restimulation. The number of ABC-specific, IFNy producing CD8+ T-cells was enriched by iterative in vitro restimulation in the presence of IL-2 and by positive selection of CD107a expressing cells. From the CD8+ enriched T-cell lines ABC-specific T-cell clones (ABC-TCC) were generated by limiting dilution. The ABC recognition pattern of the TCC was investigated by means of calcium influx kinetics analyses. *Results:* Obtained data of eight different ABC-TCC suggest that TCC can react with the drug in two ways: via the hapten-model and via the p-I model. In the former pathway, ABC is taken up by antigen presenting cells and is then stably presented to the TCC (representing an ABC processing - dependent pathway). In the latter situation, TCC are immediately activated by ABC binding from the outside to the MHC-TCR complex, leading to a Ca-Influx within 3 minutes. The fast Ca-Influx is only compatible with a processing and metabolism independent ABC recognition. *Conclusion:* As in other drug allergies (sulfamethoxazole, contrast media) the T-cell immune response to ABC occurs via a processing dependent and independent pathway. Based on the ABC model, one might investigate the primary immune response to drugs in vitro. An important point will be to investigate how an immune activation via the p-i concept is started, e.g. to what extent naïve or memory T cells as well as dendritic cells are activated by abacavir.

Stimulation of T-cells from hypersensitive patients with cystic fibrosis by sulfamethoxazole metabolites

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Poster

Background: Antigen-specific T-cells are involved in the pathogenesis of sulfamethoxazole (SMX) hypersensitivity. The aim of this study was to utilize lymphocytes from SMX hypersensitive patients with and without cystic fibrosis (CF) to explore the chemical basis of drug-specific T-cell activation. *Methods:* Lymphocytes were isolated from six patients (3 with CF), who developed delayed-type allergic reactions following SMX exposure, and incubated with SMX for 6 days. Proliferation was measured by addition of [3H]thymidine. T-cells from responding cultures were cloned by serial dilution to evaluate their phenotype, function, mechanism of antigen presentation and the involvement of metabolism in antigen presentation. *Results:* Lymphocytes from patients with and without CF were stimulated to proliferate when incubated with SMX (100 microg/ml; SI range 2-15) and nitroso SMX (80 microM; SI range 2-28). Over 5000 T-cell clones were generated from the hypersensitive patients and studied in terms of their antigen-specificity. 98 clones were stimulated with SMX (50-200microg/ml; SI range 2-39, 66% CD4+, 21% CD8+, and 11% dual positive), while 154 clones proliferated in the presence of SMX-NO (20-160microM; SI range 2-49, 87% CD4+, 10% CD8+ and 3% dual positive). In patients without CF, SMX stimulated T-cells via a direct interaction with MHC molecules. Nitroso SMX stimulated T-cells by (1) binding irreversibly to MHC and (2) a classical hapten mechanism involving antigen processing. In contrast, T-cell clones from patients with CF were stimulated with both SMX and SMX-NO. The response to SMX was dependent on metabolism by APC and intracellular adduct formation. The SMX-specific proliferative response was inhibited by the enzyme inhibitors methimazole and 1-aminobenzotriazole. Furthermore, pulsing APC for 16h generated an antigenic signal for T-cells. *Conclusion:* These results demonstrate that intracellular SMX metabolism by immune cells and subsequent protein adduct formation represents an important antigenic signal in hypersensitive patients with CF. This work was conducted in the MRC Centre for Drug Safety Science [grant number G0700654]. The project received funding from the Wellcome Trust [grant number 078598/Z/05/Z)]. AE is a PhD student funded by the Egyptian Government.

Stimulation of human T-cells with sulfonamides and sulfonamide metabolites

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Poster

Background: Exposure to sulfonamides is associated with a high incidence of hypersensitivity reactions. Antigen-specific T-cells are involved in the pathogenesis; however, the nature of the antigen interacting with specific T-cell receptors is not fully defined. The objective of this study was to explore the frequency of sulfamethoxazole and sulfamethoxazole metabolite-specific T-cells in hypersensitive patients, delineate the specificity of clones, define mechanisms of presentation and explore T-cell cross-reactivity with structurally-related sulfonamide metabolites. *Methods:* Sulfamethoxazole and sulfamethoxazole metabolite-specific T-cell clones were generated from 3 patients. Antigen specificity, mechanisms of antigen presentation and cross-reactivity of specific clones was then explored. Low-lying energy conformations of drug (metabolites) were modelled and the energies available for protein binding estimated. *Results:* Lymphocytes proliferated with parent drugs (sulfamethoxazole, sulfadiazine and sulfapyridine), hydroxylamine and nitroso metabolites. Three patterns of drug (metabolite) stimulation were seen: 44% were sulfamethoxazole metabolite-specific; 43% were stimulated with sulfamethoxazole metabolites and sulfamethoxazole, 14% were stimulated with sulfamethoxazole alone. Most metabolite-responsive T-cells were stimulated with nitroso sulfamethoxazole-modified protein via a hapten mechanism involving processing. In contrast to sulfamethoxazole-specific clones, that were highly specific, greater than 50% of nitroso sulfamethoxazole specific clones were stimulated with nitroso metabolites of sulfapyridine and sulfadiazine, but not nitrosobenzene. Phar-

macophore modelling illustrated that the summation of available binding energies for protein interactions and the preferred spatial arrangement of atoms in each molecule, determine a drug's potential to stimulate specific T-cells. *Conclusion:* These data demonstrate that nitroso sulfonamide metabolites form potent antigenic determinants for T-cells from hypersensitive patients. T-cell responses against drug(metabolites) bound directly to MHC or MHC/peptide complexes may occur through cross-reactivity with the haptenic immunogen. This work was conducted in the MRC Centre for Drug Safety Science [grant number G0700654]. The project received funding from the Wellcome Trust [grant number 078598/Z/05/Z)]. JLC is a PhD student funded by the Mexican Government.

Sulfamethoxazole antigenicity, immunogenicity and co-stimulatory signalling: evidence for the formation of a functional antigen through immune cell drug metabolism

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Poster

Background: Sulfamethoxazole (SMX) is metabolized to a protein-reactive nitroso intermediate (SMX-NO), which has been implicated in the pathogenesis of T-cell-mediated hypersensitivity reactions. The aim of this study was to characterize functional antigens in immune cells and define the complex relationship between protein adduct formation, cell death, dendritic cell co-stimulatory signalling and the stimulation of specific T-cells. *Methods and Results:* Formation of SMX protein adducts in mouse (splenocytes and dendritic cells) and human (Lymphocytes, B-cell lines and dendritic cells) cells was (1) dose- and time-dependent, (2) detectable at non-toxic concentrations and (3) blocked by the enzyme inhibitors methimazole and 1-aminobenzotriazole. Adduct formation above a specific threshold was associated with the induction of necrotic cell death and SMX-NO-treated dead cells were a potent signal for dendritic cell co-stimulatory signalling and cytokine secretion. Dendritic cells and splenocytes cultured with SMX for 6-16 h, the time needed for metabolism and adduct formation, stimulated the proliferation of T-cells from SMX-NO sensitized mice. The response of sensitized T-cells to SMX, but not SMX-NO, was blocked by enzyme inhibition. Dendritic cells cultured with SMX and adoptively transferred to naive recipient mice stimulated an immune response; however, specific T-cells were stimulated with SMX-NO, not the parent drug. Finally, SMX-pulsed autologous human antigen presenting cells stimulated lymphocytes and 82% of SMX-NO specific T-cell clones from hypersensitive patients. Enzyme inhibition decreased SMX adduct formation and the stimulation of T-cell clones. *Conclusion:* This study shows that immune cells metabolize SMX; subsequent irreversible binding to intracellular proteins generates a functional T-cell antigen in human and mouse models. Adduct formation above a threshold stimulates necrotic cell death, which provides a potent maturation signal for dendritic cells. This work was conducted in the MRC Centre for Drug Safety Science [grant number G0700654]. The project received funding from the Wellcome Trust [grant number 078598/Z/05/Z)]. AE is a PhD student funded by the Egyptian Government

Involvement of drug-specific CD4+ and CD8+ T-cells in trimethoprim-mediated hepatitis

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Poster

Background: Drug-induced liver injury is a cause of significant patient mortality and morbidity; hepatic adverse events are a major cause of drug withdrawal. Increasing evidence indicates that the release of endogenous mediators from dead or dying cells stimulates an innate immune response; however, to date, the involvement of T-cells in drug-induced liver injury has not been defined. In this study, the nature of the T-cell response in a patient who experienced severe hepatitis following trimethoprim (TMP) exposure was delineated. *Clinical details:* The patient, a 20 year old female, developed a mild rash two weeks after initiating trimethoprim treatment. In the fourth week after trimethoprim onset she was admitted to hospital with worsening rash, fever, eosinophilia and aspartate transaminase levels above 6000IU/L; she was subsequently transferred to a specialist liver transplant unit. The patient was discharged eight days after admission and liver function tests were normal at two month follow-up. *Methods and Results:* Lymphocytes from the hypersensitive patient were stimulated to proliferate with trimethoprim (3801±1174.4 cpm, [no drug]; (25368±3900.5 cpm, [trimethoprim 25 μg/ml]; $P<0.05$). 35 and 14 CD4+ and CD8+ T-cell clones that proliferated and secreted high levels of INF-gamma, TNF-alpha & IP-10 following trimethoprim stimulation were generated from the allergic patient. Interestingly, CD4+, but not CD8+, clones killed autologous target cells, assessed using a 4h [51Cr] release assay and by measuring a transient increase in CD107a expression. Trimethoprim activated specific T-cell receptors on T-cell clones via two pathways: the first involved direct drug binding to MHC and the T-cell receptor; the second involved protein complex formation and antigen processing. Processing-dependent trimethoprim presentation to T-cells was blocked with the enzyme inhibitors methimazole and 1-aminobenzotriazole. Trimethoprim-specific T-cells were highly specific in terms of drug structure; clones were stimulated with trimethoprim, pyrimethamine, and diaveridine, but not the other structurally-related compounds. *Conclusion:* These data indicate that T cells responsive towards the parent drug and drug metabolites are present in the circulation of a patient with trimethoprim-mediated hepatitis. This work was conducted in the MRC Centre for Drug Safety Science [grant number G0700654]. SEG is a PhD student funded by the Egyptian Government.

Establishment of an HLA-typed cohort to elucidate the cellular and chemical basis of drug hypersensitivity reactions

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Poster

Background: Drug hypersensitivity reactions represent a major clini-

cal problem. Furthermore, due to the idiosyncratic nature of the majority of drug hypersensitivity reactions, they only become apparent post-marketing; subsequent drug withdrawals result in the restriction of otherwise effective therapeutic agents. Recently, significant genetic associations have been identified within the MHC region, for drugs such as abacavir (HLA-B*5701), flucloxacillin (HLA-B*5701), carbamazepine (HLA-B*1502), and ximelagatran (HLA-DRB1*0701). For abacavir, patients are now genotyped for HLA-B*5701; limiting drug exposure to HLA-B*5701 negative individuals has effectively reduced the incidence of hypersensitivity. The objective of this study was to establish an HLA typed cohort of frozen human lymphocytes from 400 healthy drug-naive volunteers to explore drug-specific T-cell responses. *Methods:* Currently 310 blood samples have been processed; of these, genotyping has been performed on 165. A panel of immunological assays (proliferation, ELIspot, CD107a) have been utilized following 14 day drug exposure to monitor drug-specific T-cell responses. Furthermore, drug-specific T-cell clones from volunteers expressing appropriate HLA molecules will be used to analyze the cellular phenotype and functionality. *Results:* From the 165 genotyped samples, 41 volunteers were found to express HLA-DRB1*0701 (ximelagatran; 4 homozygotes, 37 heterozygotes) and 13 volunteers expressed HLA-B*5701 (abacavir and flucloxacillin; 1 homozygote, 12 heterozygotes). In preliminary studies, lymphocytes from a HLA-B*5701 positive volunteer were stimulated to proliferate and secrete IFN-gamma following abacavir exposure. The antigen-specific response was dependent on the 14 day enrichment step with drug and IL-2. Lymphocytes from HLA-B*5701 negative volunteers were not stimulated with abacavir. Fourteen abacavir-specific T-cell clones were successfully generated from the HLA-B*5701 positive volunteer. *Conclusion:* Experiments are on-going to characterize the phenotype and functionality of the clones, and to define the mechanism(s) of antigen presentation. Furthermore, partly matched and unmatched antigen presenting cells identified from the HLA typed cohort are to be used to define the specific involvement of different HLA types in antigen-specific T-cell stimulation. This work was conducted in the MRC CDSS [grant number G0700654]. The project received funding from AstraZeneca.

Drug specific T cells in delayed allergic drug reactions

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Poster

Delayed allergic drug reactions are mediated by T cells and mostly affect the skin. They comprise several diseases ranging from the frequent and benign maculo-papular exanthema (MPE) to the severe and rare toxic epidermal necrolysis (TEN). CD8+ CTLs are involved in the pathophysiology of severe drug reactions such as TEN. However, their contribution to the onset of non severe drug reactions such as MPE is still under discussion. To get insight into the pathophysiology of these reactions, we studied the presence of drug specific T cells in the blood and skin of drug allergic patients. All patients included in this study had a well known history of non severe drug-induced delayed allergic reaction and had positive skin patch-tests to the drug. Drug-specific T cells can be found in the blood of patients using both secondary proliferation assays and cytokine release tests as ELIspot assays. Analysis of circulating-drug-specific T cells show a high frequency of drug specific IFNγ, IL-17 and Granzyme B(GrB) secreting CD8+ T cells ([mean: 59 SFC /106 PBMCs+/- 21 for IFN-γ],[mean: 58 SFC /106 PBMCs+/- 8 for IL-17], [mean: 37 SFC /106 PBMCs+/- 5 for GrB]). Importantly, no IFN-g the kinetics of T cell infiltration in the skin lesions have been done using skin patch-tests like an experimental model of drug-induced skin inflammation. Results revealed a recruitment of activated CD8+ Tc1 cells (IFN-g+, perforin+, GrB+) in the epidermis as early as 12 hrs after drug skin application and associated with keratinocyte apoptosis. Furthermore, skin recruitment of CD4+ T cells and other leucocytes occurred later (48 hrs) and paralleled the down-regulation of CD8+ T cell activation. These data indicate that: i) drug-primed CD8+ Tc1 cells are involved in the development of skin inflammation in non severe allergic drug reaction; ii) drug specific CD8+ Tc1 (IFNγ+, GrB+) can be detected in peripheral blood of allergic patients using Elispot assays. Our results demonstrate that CD8+ T cells are involved in both the severe and non severe forms of drug-induced skin allergy. Moreover, the measurement of IL-17, GrB and IFN-γ release after drug restimulation using ELISPOT assay might be a useful in vitro tool for detection of drug specific T-cell and may be an interesting alternative to improve the diagnosis of drug allergy

Characterization of piperacillin-specific T-cell clones from hypersensitive patients with cystic fibrosis

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Poster

Background: Piperacillin (PIP) exposure in patients with cystic fibrosis is associated with a high incidence of delayed-type hypersensitivity reactions. In this study, the nature of the PIP-specific T-cell response was delineated by means of in vitro stimulation of lymphocytes and T-cell clones. *Methods:* Lymphocytes were isolated from four patients that developed maculopapular exanthema and fever (+/- arthralgia or flu-like symptoms), following PIP exposure and incubated with PIP for 6 days. Proliferation was measured by addition of [3H]thymidine. T-cells from responding cultures were cloned by serial dilution to evaluate their phenotype, function (proliferation, cytokine secretion and cytolytic activity), mechanism of antigen presentation and cross reactivity with structurally related compounds. *Results:* Lymphocytes from allergic patients were stimulated to proliferate with PIP ([control; 803±250 cpm], (1mM PIP; 4554±438 cpm)]. Lymphocytes from non-hypersensitive patients were not stimulated. CD4+, CD8+ and CD4+CD8+ T-cell clones, which proliferated, killed target cells and secreted high levels of IL4, IL5, IFN-γ, and MIP-1β following PIP stimulation, were generated from the hypersensitive patients. The proliferative response was (1) highly specific

in terms of drug structure - T-cells were stimulated with PIP, but not structurally-related drugs; and (2) dependent on drug-protein conjugation. *Conclusion:* These data indicate that T cells responsive towards the PIP protein conjugates are present in the circulation of hypersensitive patients.

Characterization of piperacillin albumin binding sites in vitro and in patients with cystic fibrosis, and T-cell responses to hapten-modified protein

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Poster

Background: Patients with cystic fibrosis require high dose i.v. antibiotics, such as piperacillin or ceftazidime, for the treatment of pulmonary infections. Unfortunately, 20-30% of exposed patients develop clinical manifestations of hypersensitivity, which results in drug withdrawal. We have recently demonstrated that covalent modification of protein with piperacillin is an obligatory step for the stimulation of drug-specific T-cells. The objective of this study was to define site specific modifications of the model protein human serum albumin with piperacillin in vitro, and in vivo in piperacillin exposed patients, and investigate the capacity of piperacillin protein conjugates to stimulate lymphocytes and T-cell clones from hypersensitive patients. *Methods:* Piperacillin was incubated with human serum albumin for 1 h – 3 days (the length of an in vitro proliferation assay), and specific amino acid modifications were characterized using sophisticated mass spectrometry. Furthermore, adducts formed in the plasma of patients exposed to piperacillin were characterized. The ability of a piperacillin albumin conjugate (modified at a drug:protein ratio of 10:1) to stimulate lymphocytes and CD4+ and CD8+ T-cell clones from 3 piperacillin hypersensitive patients was measured in vitro using [3H]thymidine. *Results:* Piperacillin was found to conjugate albumin in a time- and concentration-dependent manner, via two chemical moieties, at 7-12 lysine residues. Modification of other amino acids residues was not detectable. A similar pattern of reactivity was observed when piperacillin was cultured with lymphocytes and T-cell clones in medium supplemented with 10% human AB serum. Furthermore, modification of two lysine residues on albumin at positions 190 and 541 was detected in plasma of drug exposed patients. Piperacillin-conjugated albumin stimulated lymphocytes from hypersensitive patients and 60 CD4+ and CD8+ piperacillin-specific T-cell clones. *Conclusion:* These data show that T cells from hypersensitive patients are stimulated with a piperacillin albumin conjugate modified at up to 12 specific lysine residues. This work was conducted in the MRC Centre for Drug Safety Science [grant number G0700654]. SEG is a PhD student funded by the Egyptian Government.

Evaluation of drug specific lymphocyte proliferation in Stevens-Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN) to lamotrigine

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Poster

Prior studies using the lymphocyte transformation test (LTT) suggest that drug specific T-cells are less often found in SJS and TEN than in other types of reactions, depending on timing of sampling (acute versus late) and regulatory T cells (T-reg) (1). We explored drug specific reactivity in vitro and the possible role of T-reg in well-defined groups of patients who reacted to the same drug: lamotrigine (LTG). *Patients and methods:* With SJS/TEN cases provided by RegiSCAR, the following groups were studied: controls not exposed to LTG (n=11), patients exposed to LTG without reaction (6), patients who developed a rash to LTG (6), acute phase samples (10) and post-recovery samples (13) from SJS/TEN to LTG. LTT was performed by measuring H3-thymidine incorporation after 3 days incubation with PHA, LTG (10 µg/ml) or medium. Stimulation index ≥ 2 was considered positive. In 16 cases LTT was redone after depletion of T-reg by FACS sorting (elimination of CD4+CD25 bright cells). *Results:* Patients with SJS/TEN differed from those with milder eruptions by the absence of reactivity in lymphocytes obtained during the acute phase and a low number (3/13) of positive LTT on later samples. We observed a trend, of borderline significance, for an increased response (in late samples only) after depletion of T-reg, but with SI remaining < 3. Positive LTT Stimulation index (mean± SD) Unseparated T-reg depleted Unseparated T-reg depleted (4/ group) Healthy controls 1/11 ND 1.49 ± 0.44 ND Exposed controls 0/6 0/4 1.48 ± 0.39 1.15 ± 0.1 Rashes late samples 3/6 2/4 3.4 ± 3.2 1.73 ± 1.1 SJS/TEN early 0/10 0/4 1.27 ± 0.41 1.2 ± 1.1 SJS/TEN late 3/13 3/4* 1.54 ± 0.50 2.1 ± 0.6** * Including 2 cases negative before sorting ** p=0.04 (unpaired t test) compared to SI before depletion. *Conclusion:* At variance with a recent study we did not find reactive cells in early phase of SJS/TEN, may be because they have been directed to the skin. On the other hand our results confirm in late SJS/TEN samples a low rate of positive LTT and a borderline increase in reactivity after depletion of T-reg. This is not enough to conclude that T-reg are the principal cause for negative reactivity (LTT or skin tests) in patients surviving SJS/TEN. Other in vitro assays than those testing proliferation should be evaluated, before raising the hypothesis that specific cells often disappear by undergoing apoptosis during the reaction.

Reference: 1)Takahashi R, Kano Y, Yamazaki Y, Kimishima M, Mizukawa Y, Shiohara T J Immunol 2009;182:8071-9.

The pathogenesis role of HLA and TCR in SJS/TEN

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Oral communication

Backgrounds: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous adverse reactions (SCARs), which may involve MHC-restricted activation of cytotoxic T lympho-

cytes (CTLs). This is supported by our genetic findings that HLA-B*1502 is strongly associated with carbamazepine-SJS/TEN, and HLA-B*5801 with allopurinol-SCARs. Recently, we also found that granulysin is a key molecule responsible for the disseminated keratinocyte death in SJS/TEN. However, how HLA-B molecules interact with small drugs to activate CTLs in SCARs, remains unclear. *Materials and methods:* We hypothesized that HLA-B*1502/5801 expressed on keratinocytes, possess higher affinity to the particular drugs, and are able to present the compounds to specific T cell receptors (TCRs) of CTLs, leading to granulysin-mediated cell death in SJS/TEN. In this study, we generated stable clones expressing HLA-B alleles, obtained PBMC from SCARs patients, and expanded drug-specific CTLs in vitro. *Results and conclusion:* In the presence of dug, CTLs showed strong cytotoxicity against target cells expressing HLA-B*1502 or B*5801, but not cells transfected with other HLA-B mutants. Granulysin was predominately produced by the in vitro expanded CTLs, resembling the observation in skin lesions of SJS/TEN. The cytotoxicity of CTLs could be abolished by anti-HLA class I antibody but not anti-HLA class II antibody. In addition, our data also suggested that activation of CTLs by HLA-B/drug is through antigen-no processing pathway. Furthermore, CDR3 spectratyping and quantitative PCR analyses of TCR repertoire revealed restricted TCR usage in SJS/TEN. The same nDn sequence of immunodominant CDR3 region have been identified in the in vitro expanded CTLs, as well as in the peripheral blood of SJS/TEN patients. In conclusion, our data demonstrated that conserved HLA-B and restricted TCR usage are the key initiators in the pathogenesis of SJS/TEN.

Fluctuation of blood and skin plasmacytoid dendritic cells in drug-induced hypersensitivity syndrome

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Oral communication

The precise mechanism underlying the reactivation of viruses in DIHS/DRESS remains unknown. Human plasmacytoid dendritic cells (pDCs) are a subset of leukocytes capable of producing large amounts of interferon (IFN)-α/β, which enables neighboring cells to resist viral infection by differentiating them into mature DCs or mature B cells to produce IgG. In this study, we sought to determine the distribution of pDCs in the blood and the skin in patients with DIHS and discuss the possibility whether pDCs are involved in the reactivation of viruses in DIHS. The percentages of lineage-CD123+ HLA-DR- pDCs in PBMCs were low especially around the period of reactivation of viruses, and those in DIHS were lower than those in healthy subjects and in patients with generalized maculopapular drug eruption. In addition, a significant number of CD123+ CD16- pDCs were detected in the dermis of patients with DIHS, but such an infiltration was not observed in other patients with generalized maculopapular drug eruption and healthy donors. This data suggests that pDCs in the circulation might accumulate in the skin and subsequently the storage of pDCs in circulation decreased. The rapid increase of pDC in the blood after treatment of DIHS may be involved in the occurrence of autoimmune diseases, such as type 1 diabetes mellitus, which can eventually develop in high frequency after DIHS.

Peptide specificity of a iodinated radio contrast medium reacting CD8+ T cell clone

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Poster

Introduction: Since "pi-acting" drugs (according to the pharmacological-concept) are not supposed to primarily prime drug reacting T cells, such cells must have been previously primed by a former antigen. As a proof of concept study, our goal is the sequence identification of the original peptide, involved in the priming of a drug reacting T cell clone (TCC). *Methods:* A Iomeprol reactive TCC had been chosen. As this clone was CD8+ and highly cytotoxic, 51Cr-release assays were performed with autologous EBV-Blcl loaded with peptides of a positional scanning synthetic combinatorial libraries (PS-SCL). This PS-SCL is composed of 171 mixtures of 9mer peptides, where one residue is defined at one given position and the rest is random. Deconvolution of the libraries leading to positive cytotoxicity gives sequences of cross-reactive peptides. *Results:* 51Cr-release assay was done in triplicate and repeated 3 times in order to achieve statistical relevance. For all nine positions of the 9mer peptide, the mean of the specific lysis from the 19 possible amino-acids (20 minus Cysteine) was calculated. Residues exceeding mean plus standard deviation were considered likely present residue belonging to the sequence of the priming peptide. One to four possible residues were identified for each position (mean 2.7 residues). Mathematical combinations of the possible residues lead to a number of 5184 possible peptides, which represents an important reduction compared to the original 3.22x1011 possibly combinatorial peptides. *Conclusion:* Most of the identified 5184 possible sequences do not exist in nature and result only from mathematical combination. Screening of transcriptome databanks should identify the most promising peptides among these sequences, which will be tested. Finally the HLA restriction of the peptide as well as the Iomerol reactivity will be compared.

Diagnostic tools in vivo

Improved preparation method and stability analysis of the penicillin minor determinant benzyl-penicilloate

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Poster

Background: Penicillin skin testing with the major determinant (benzyl-penicilloyl) and minor determinant mix (penicillin G, benzyl-penicilloate, and benzyl-penilloate) is the most reliable tool for diag-

nosing a penicillin allergy mediated by IgE. Benzyl-penicilloate and penilloate can be produced by several methods which are long and difficult (Clarke 1949; Levine 1964; Voss 1966; Levine and Redmond 1969; Ressler, Neag et al. 1985). The objective of the study was to optimize the production of benzyl-penicilloate by developing a method of production that is faster and simple. The second goal was to examine the stability of the benzyl-penicilloate in aqueous solution by nuclear magnetic resonance (NMR). *Methods:* Penicillin G sodium was dissolved in distilled water. NaOH 1N was used to increase the pH. A constant pH of 11 or 12 was maintained for 60 or 120 minutes to optimize the production of benzyl-penicilloate. HCl 0.2N was then added to decrease the pH to 7. The mixture was then lyophilized and stored in a desiccator at 4°C. The product (benzyl-penicilloate) was verified by NMR and analyzed for the different diastereoisomers of benzyl-penicilloate in aqueous solution. Detection threshold of diastereoisomers by NMR method used (500 MHz 1D NMR with 128 scans) was estimated to be at ~0.2 %. *Results:* The optimal pH and time in the alkaline hydrolysis are pH 11 for 120 minutes or pH 12 for 60 minutes. Three out of the four possible diastereomers of benzyl-penicilloate were produced at detectable levels (57% 3S, 5S, 6R-benzyl-penicilloate; 42% 3S, 5R, 6R-benzyl-penicilloate; 1% 3S, 5S, 6S-benzyl-penicilloate; >0.2% 3S, 5R, 6S-benzyl-penicilloate). However, after 24 hours in aqueous solution at room temperature, 86% were 3S, 5S, 6R-benzyl-penicilloate; 11% 3S, 5R, 6R-benzyl-penicilloate; 3% 3S, 5S, 6S-benzyl-penicilloate; >0.2% 3S, 5R, 6S-benzyl-penicilloate. No further breakdown of penicilloate to other products such as benzyl-penilloate was noted. *Conclusion:* This modified alkaline hydrolysis technique is an effective and rapid method of producing benzyl-penicilloate. The benzyl-penicilloate consisted of three different diastereoisomers that changed in relative proportions when maintained for 24 hours at room temperature. Skin testing with these products is in progress.

Nonirritating concentration for skin testing with cephalosporins

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Poster

Skin testing with drugs should be made at the highest concentration of drug that would not elicit an irritant skin test response in an adequate number of healthy control subjects . If for diagnostics on the penicillin there is agreement in the nonirritating concentrations, we can't say the same thing for cephalosporins. Empedrad R JACI 2003 advised to perform prick and intradermal testing using the concentration of 10 mg/ml for cefotaxime, cefuroxime ceftazidime and ceftriaxone and 33 mg/ml for cefazolin; 1-2 mg/ml was recommended for prick and intradermal tests by Torres MJ Allergy 2003, and later 2 mg/ml by Blanca M Allergy 2009. We aimed to ascertain if the concentration of 20 mg/ml is nonirritating for intradermal skin tests for cephalosporins. In our Center since 1988 in the cutaneous diagnosis of betalactams we have been performing skin and intradermal tests with PPL (5 x 10-5 mMol/l) and MDM (5 x 10-2 mMol/l), benziylpenicillin (10,000 IU/ml), amoxicillin-clavulanic (20 mg/ml) and cefuroxime (20 mg/ml). The same concentration of 20 mg/ml has been used for all the other cephalosporins and the betalactams that from time to time are added if suspected of being the cause of adverse reaction. We have conducted a survey on our database, from January 2000 to June 2009 and the first data reported here are worthy of significance. In our experience, the prick and intradermal tests for cephalosporins at a concentration of 20 mg/ml were not irritating with the exception of cefepime (and aztreonam). So using this concentration to perform skin tests would increase the sensitivity of the test and can diagnose even those patients who would otherwise have had negative results. The fact that cefepime and aztreonam showed to be irritating at the concentration of 20 mg/ml was due most probably to L-Arginine contain in the lyophilized powder. In fact, these betalactams in our center, continue to be tested at a concentration of 2 mg/ml. At the current stage of our investigation we can say that some cephalosporins are not irritating to the concentration of 20 mg/ml, but most likely this rule does not apply to all members of the family. This should encourage us to move forward in our research about nonirritating concentrations, although for some less frequently used cephalosporins we'll need to combine the results of several Centers that deal with this type of diagnosis.

Skin test predictive value on the proton pump inhibitors allergy

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Poster

Background: The proton pump inhibitors (PPI), are widely used in the treatment of peptic ulcer and gastroesophageal diseases. The incidence of the skin rashes induced by PPI is less then 0.5%. Only a few cases of allergic anaphylactic or erithrodermic reaction to omeprazole, pantoprazole and lansoprazole were recorded. Additionally some cases of cross-reactivity within PPI have been reported and cross-reactivity between PPI and others imidozoles (metronidazole, tiaconazole, bifonazole, econazole, miconazole) have been postulated. The aim of our prospective study was to verify sensitivity (Se) and specificity (Sp) of skin prick test (PT) and intradermal test (ID) in the diagnosis of PPI allergy. *Methods:* We present the preliminary data of a study performed in 26 patients, 20 female and 6 male, with history of adverse reactions to PPI. The mean age of our patients was 63,6 years. PT with omeprazole, esomeprazole (40 mg/ml), pantoprazole (20 mg/ml) and lansoprazole (30 mg/ml), and ID at the concentrations of 0.02 and 0.2 mg/ml for esomeprazole and omeprazole and 0.04 and 0.4 mg/ml for pantoprazole were performed in all patients. Then all patients underwent to a single blind placebo controlled challenge test (CT) with the culprit PPI. 4 allergic patients underwent also to a CT with an alternative PPI. *Results:* Ten (39%) patients have reacted to esomeprazole, 6 (23%) to omeprazole, 6 (23%) to pantoprazole and 4 (15%) to lansoprazole. The symptoms reported were urticaria (54%), angioedema (15%), erythema (15%), dyspnea (12%) and hypotension (4%). PT were negative; ID were positive in 3 patients for the culprit drug. CT were positive in 4 cases (2 to esomeprazole, 1 to lansoprazole and 1 to pantoprazole) but only in 2 cases ID were positive. The predictive capacity of skin test was associated in our cohort to a low Se of 50% but to a high Sp of 95%. Moreover the positive predictive value (PPV) and the negative pre-

dictive value (NPV) were respectively 66% and 91%. *Conclusions:* Our results suggest a poor predictive capacity of a positive skin test, that confirm the need to perform CT. Furthermore we didn't find cross-reactivity within PPI, in fact the 4 allergic patient tolerated others PPI. More numerous cohort study is necessary.

The role of skin testing and drug provocation test in the diagnosis of non-immediate reaction to radio contrast media

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Poster

Background: The frequency of non-immediate reactions to iodinated contrast media (CM) seems to have increased in the last decade, being skin testing the method usually used for diagnosis. The sensitivity of skin testing seems to be lower than 50%, and this has not been confirmed by drug provocation test (DPT). *Objective:* To evaluate the role of skin testing and DPT in the diagnosis of patients with non-immediate hypersensitivity reactions to CM. *Methods:* Patients (N=100) with a non immediate hypersensitivity reaction with skin involvement after CM administration were studied. Skin prick and intradermal testing with delayed readings, were done with different CM (Iobitridol, Iomeprol, Iodixanol, Iohexol, Ioversol, Iopramide and Ioxaglate). DPT was done in those cases with skin test negative for confirming the diagnosis and in those with skin test positive to search for an alternative CM. Skin biopsy was obtained from positive skin tests and positive DPT. *Results:* Skin tests were positive in 18 (18%) (10 to Iomeprol, 4 to Iodixanol, 2 to Iopramide and 2 to Ioxaglate) and negative in 80 (80%) cases. From those negative 28 patients (34.15%) developed 36 positive DPT with CM (26 to Iodixanol, 6 to Iomeprol, and 6 to Iohexol). This indicates that we could confirmed the diagnosis in 46% of the patients evaluated, 39% by skin testing and 60% by DPT. A total of 106 DPT was done (52 to to Iodixanol, 34 to Iomeprol, 12 to Iohexol, 6 to Dimeglumine and 2 to Iobitridol). Forty-four (41.5%) DPT were positive (68.2% to Iodixanol, 18.2% to Iohexol and 13.6% to Iomeprol), being these differences significant ($p<0.001$). Conclusion: Skin test sensitivity with CM is lower than previously thought diagnosing less than 50% of the patients. The sensitivity of the test depends on the CM used being higher with Iomeprol. On the other hand Iodixanol was the CM most frequently inducing a positive DPT.

Diagnosis of immediate hypersensitivity reactions to contrast material: the CIRTACI study

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Oral communication

Background: The CIRTACI study is a French prospective multicenter trial which aims at elucidating the mechanisms of immediate hypersensitivity reactions to contrast media (CM) *Methods:* Thirty-one investigation centres were set in university hospitals, each conducted by a radiologist and an allergologist. Patients who reacted within one hour of injection of either an iodinated (ICM) or a gadolinated contrast media(GCM) had records of the name of the CM and the clinical signs. They had early measurements of plasma histamine and tryptase, and were skin-tested later by Prick tests and intradermal tests (IDT) with diluted and pure solutions of ten ICM or 5 GCM, as appropriate. Allergic hypersensitivity was diagnosed by an expert panel, and based on the clinical symptoms, positive skin tests (doubling of the injection papula) to the culprit CM or positive tryptase, and non-allergic hypersensitivity where skin tests and tryptase were negative. *Results:* The study lasted for 4.5 y. Two hundred and sixty-seven patients were included after ICM (mostly non-ionic ICM) injection and 48 after GCM. Cutaneous-mucous reactions (grade 1) occurred in 180 patients, mild hypotension or dyspnoea (grade 2) in 85, severe hypotension or bronchospasm (grade 3) in 43, and cardiac arrest (grade 4) in 6. The frequency of positive skin tests to the culprit CM increased with the clinical severity of the reaction, as well as the frequency of elevated concentrations of mediators. Most of the patients with positive IDT to the culprit CM had negative IDT to several other CM. *Conclusion:* The mechanisms of immediate reactions to CM can be elucidated through biochemical and skin tests, thus allowing counselling for subsequent radiological procedures.

Fixed drug eruption to ibuprofen

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Poster

Background: Fixed drug eruption due to ibuprofen has been rarely described. *Materials and methods:* Patient without allergic history,ten days after tratment with ibuprofen and omeprazol, presented lesions erythematosus-violaceous in the left forearm.The lesions disappeared after seven days leaving a residual hyperpigmentation. *Results:* Topical challenged by occluded patch test with ibuprofen 5% in petrolatum on residual cutaneous lesion,resulted positive-48 hours and 96 hours later an erythematosus-violaceous lesion (2x2 cm). Oral challenged test with omeprazol was negative. *Conclusions:* We present an case of fixed drug eruption to ibuprofen, diagnosed by positive epicutaneous test on residual lesion.

Patch testing in severe cutaneous adverse drug reactions: a study of 111 patients

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Oral communication

Background: Patch testing may help to assess the culpability of a drug in severe cutaneous adverse drug reactions (ADRs). Our aim was to study patch testing on the following ARDs: acute exanthematic pustulosis (AGEP), drug reaction eosinophilic hypersensitivity syndrome (DRESS), Fixed Drug Eruption (FDE), Stevens-Johnson syndrome

(SJS) and toxic epidemal necrolysis (TEN). *Material and methods:* Patients with ADRs and patch tested with the suspected drug from January 1996 to December 2008 were retrospectively included. Patch test were performed with the suspected drug at 1% or 10% and with a standard series of drug, 3 to 8 weeks after recovery. For each patient, drug responsibility was assessed by the official method of the French pharmacovigilance. A score of 0-3 was attributed to each drug taken during the month before the first cutaneous sign. *Results:* 111 patients with ADRs were included: 32 had AGEP, 28 DRESS, 12 FDE and 39 SJS/TEN. Mean age was 53 years (21-89 years), sex ratio 1.3 (F/M). 71 patients had more than one suspected drug, patch tests were positive for 41% (46/111) of the patients: AGEP (63%), DRESS (50%), FDE (17%), SJS/TEN (26%). The proportion of positive patch tests was significantly higher for AGEP than SJS/TEN, p<0,003. 44 patients had a positive patch test with the drug of the highest imputability score (96%). For the 71 patients who had 2 or more suspected culprit drugs, patch tests were positive in 38% (27/71) and with the highest immutability score in 81% (22/27). No ADRs relapse or severe side effects were reported. The majority of positive Patch tests confirmed the highest drug imputability score, and pointed the culprit drug in one third of the cases when there were 2 or more suspected. *Conclusions:* Although a weak sensitivity patch testing with the suspected drug is a very useful tool in assessing the drug responsibility in ADRs. Its sensitivity is higher with AGEP than SJS/TEN. Nevertheless, a negative patch test will not completely eliminate the suspected culprit drug.

Diagnostic drug provocation tests: post-evaluation of negative paediatric patients

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Poster

Background: Drug hypersensitivity (DH) is related to the prescription of alternative drugs, in many cases more costly and with a less favourable safety profile. Diagnosis is largely based on in vivo tests such skin tests and drug provocation tests (DPT). The aim of this study was to evaluate the therapeutic options used in paediatric patients with a previously negative diagnostic DPT (with the culprit drug - CD). Possible causes related to the non-use of the investigated drug were analysed. *Methods:* 50 paediatric patients with a previous negative DPT to a beta-lactam antibiotic (AB) or NSAID's performed 1 year before evaluation were included. A telephonic questionnaire was used to assess if the tested drug was used after DPT and the reasons for the non-use. *Results:* 10 patients could not be contacted. 40 patients were evaluated (23 female), with a medium age of 11.53±5.15 years, mean age of reaction 4.94±4.62 years and mean age at DPT of 8.05±4.73 years. AB's were implicated in 63% of sample and NSAID's in 38%. Questionnaires were applied mostly to mothers (83%); the mean age of the interviewed was 41.14±10.54 years. The majority of the questioned people (75%) had at the maximum the second stage of basic education (level 2 of ISCD) and 78% had an inferior or intermediate professional level. Everyone interviewed remembered the DPT result, but only 78% remembered the exact name of the drug. 10% of caregivers still think that the child is allergic to the drug. 53% of the patients have taken the drug (48% antibiotics and 52% NSAID's, p=0.041); no patients reported new hypersensitivity reactions. From the remaining 19, 45% didn't take the drug because it wasn't needed, 35% because of fear of another reaction and 25% because doctors chose another. Lowest educational level were related to the non-use of the studied drug (p=0.009) and fears of a new reaction (NS), especially AB (NS). *Conclusion:* The investigated drugs as well as the PT result were remembered by a large majority of the sample. However, 10% still believes in the persistence of DH. The reasons for not taking the drug were more related to the absence of indication, rather than fears. These fears were more common in caregivers with lower educational levels. Our results support the usefulness of DPT to clarify suspicious DH reactions to NSAID's and AB as most paediatric patients will have the requirement to use these drugs in the near future; the negative DPT are predictive of a posterior tolerance.

Skin testing with infliximab to assess immunogenicity: description of two cases

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Poster

Background: Infliximab is a chimeric monoclonal antibody against TNF-α useful in the treatment of many chronic inflammatory diseases. Adverse reactions during infusion have been reported and related to the development of specific IgG and non-IgG antibodies. Furthermore, the relationship between therapy interruption and risk of infliximab infusion reaction has been already described. Exact diagnostic tool in this field of drug allergy has not been yet elucidated in order to identify subjects at risk of reaction. *Materials and methods:* We evaluated the usefulness of skin testing to assess the immunogenicity of infliximab in two patients during a second course of therapy, after a period of treatment interruption. Serum samples were also taken at baseline and before each infusion to detect non isotype-specific (Immundiagnostick AG) and IgE (Phadia Diagnostics) anti-infliximab antibodies (ATI). Skin testing was carried out with the commercial infliximab preparation; the starting concentrations were 1:1000 for the prick test and 1:10000 for the intradermal test. In both prick and intradermal testing a minimum wheal area of 3 mm in diameter or an increase of area >3 mm was considered as positive compared to a negative response to the saline control. Undiluted infliximab preparation did not show any irritating effect in non infliximab-exposed control subjects. *Results:* Case 1: Skin testing with infliximab were performed before (T0) and twelve days (T1) after the first infusion of the second course of therapy. Intradermal test (IDR) at 1:10 drug dilution was positive at immediate lecture only at T1. Control with saline solution resulted always negative. Combined with skin testing results, only the serum sample collected at the moment of T1 resulted positive for non isotype-specific and IgE ATI. Case 2: At baseline both skin testing and ATI detection were negative. IDR with infliximab (1:100) showed a positive result at immediate lecture ten days after the first infliximab infusion (of the second course). A concomitant positivity of serum ATI and skin testing was also displayed. *Conclusions:* We conclude that after a period of inter-

ruption of therapy, at the beginning of a second course of treatment, the production of IgE ATI able to induce type I hypersensitivity reactions, may be monitorised by using skin testing. This appears to have a significant clinical meaning, allowing to identify potentially reactive patients and to avoide acute severe reactions.

Tolerance to systemic hydrocortisone and methylprednisolone in patients sensitized to tixocortol pivalate

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Poster

Background: Contact allergy to topical corticosteroids is well-established but frequently misdiagnosed. Epicutaneous tests are considered useful for diagnosis and cross-reactions study, and corticoids have been classified in four groups based on structural, clinical characteristics and patch tests results. Tixocortol pivalate, included in standard series, is considered one of the markers for allergy to corticoids. It is included in group A of Coopman's classification, as well as hydrocortisone and methylprednisolone, two systemic corticoids widely used in our emergency rooms. Correlation of sensitization to topical corticosteroids with tolerance to systemic ones is not well known and may require challenge tests. *Material and methods:* Patients with suspected diagnosis of contact allergy to topical corticosteroids were evaluated with a detailed clinical history. Epicutaneous tests with the suspected drug, the battery of corticosteroids and with commercial corticoids were performed. If tixocortol pivalate was positive in patch tests, prick and intradermal tests were performed to systemic corticoids from group A and if they were negative, a challenge test with the alternative drug was carried out. *Results and conclusions:* Three patients (2 male and 1 female, 43-56 y.o) with suspected contact allergy to topical corticoids were evaluated. Involved drugs were topical dexamethasone phosphate (2 cases) and topical hydrocortisone aceponate (1 case). In all cases patch tests were positive for the suspected drug as well as for tixocortol pivalate. Prick and intradermal test were performed to hydrocortisone in both patients that had negative patch test for this drug, and with methylprednisolone for the one that had a positive patch test to hydrocortisone. A controlled challenge test with the alternative group A systemic corticoid (parenteral hydrortisone and/or oral metylprednisolone) was carried out with good tolerance in the three patients. We conclude that although larger series are necessary for assessment of real cross- reactivity between tixocortol pivalate and group A systemic corticoids, a tixocortol pivalate sensitization may require a challenge study to confirm tolerance to hydrocortisone phosphate or methylprednisolone.

Drug intradermal tests: to standardize the methods is essential

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Poster

Objective: A study in the ENDA group of the European Academy of Allergy and Clinical Immunology (EAACI) has shown that none of 20 centers did and interpreted drug intradermal tests (IDT) in the same way. To determine the threshold for specificity for drug IDT it is absolutely necessary to standardize our methods in knowing the injected quantity of allergen. The objectives were from a unicentre study to compare the negative predictive value (NPV) and the specificity of IDT according 3 reading's criteria and to determine the average diameter of injection papules (Pi) obtained with 0.9% saline as it was proposed at the meeting of ENDA in October 2009. *Methods:* In patients who had a reintroduction (R) of the drug after negative IDT according to the criteria of the European Society of Contact Dermatitis (ESCD) (the diameter of the wheal read at 20 minutes (P20) ≥ twice the diameter of Pi), we re-analyzed, according to the criteria for positivity of the EACCI (P20 ≥ Pi+3 mm) and the criteria 'P20 ≥ 6 mm'. With a multicentre study, the mean diameter of Pi obtained with an injection of 0.03 ml of saline was determined. *Results:* 157 negative IDT according to the ESCD criteria, preceded 49 R. With the EAACI criteria 5 IDT were +, and 47 Pi were ≥ 6 mm. With the criteria of ESCD, EACCI or P20 ≥ 6 mm, we respectively found a NPV of 48%, 47% and 45% with a specificity of 95% for the EACCI criteria but only 65% for P20 ≥ 6mm. From 5 centres, the mean diameter of Pi with saline was 4.8 mm (3 to 7 mm). According to the injected site the mean diameter was 5.01 mm on the arm (47 IDT) and 4.63 mm on the forearm (77 IDT). *Discussion:* Compared to ESCD criteria, EAACI criteria could have a better sensitivity with an equal specificity, but the criteria "P20≥ 6 mm"cannot be used because it has a very poor negative predictive value. In contrast to previous publications, we demonstrated that injecting saline does not induce a 3 mm Pi. A prospective multicentre study is needed to determine the best way to perform and interpret immediate IDT reading or to confuse the 2 European criteria by working with a diameter of Pi of 3 mm. The injection of 0.02 ml would be the adequate volume and be less painful than 0.03 ml, especially in children but difficult to control with currently marketed syringes. A larger study is needed to determine if there is any significant difference of the diameter of Pi on the arm, the forearm or the back.

Evaluation of a practice of managing drug eruptions due to Interferon, specific to 15 cases

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Poster

Background: The management of β type cutaneous adverse drug reactions (CADR) due to interferons (IFN) is unclear and can lead to stop these highly potent drugs. The objectives were to determine the usefulness of topical corticosteroids (DC) in CADR due to IFN, the value of skin tests, the existence of cross-sensitizations and their im-

pact on the choice of replacement IFN in case of hypersensitivity. *Materials and Methods:* A retrospective analysis of all medical files of patients referred for CADR due to IFN was done, regardless of its class and the concerned pathology. *Results:* Fifteen patients (8 M, 7 F, mean age: 54 y.o.), 12 treated with IFNα and 3 with IFNb, developed generalized urticaria in 2 cases, generalized eczema in 7 cases, maculo-papular exanthema in 5 cases and severe eczema located on the injection site only in 1 case. All Patch-tests and prick-tests were negative Delayed positive reactions occurred on IDT done with the responsible IFN in 6/9 tested patients (delay from 24 h to 6 days). Two patients with delayed positive IDT had a subcutaneous reintroduction of this IFN, reinducing the CADR in 1 case, tolerated with DC in the 2nd case. Among the 7 patients who were tested for several IFN, cross-reactions were observed in 4 cases (within the same class of IFN or not). In 8/15 cases, treatment was stopped, in 4 cases it was replaced by another IFN which had negative results on IDT with a good tolerance. In 3/7 cases the responsible IFN was continued, DC and antihistaminic (Anti-H1) could control the CADR. In 2 cases, UVBTL01 gave an improvement and in 1 case, the application of topical tacrolimus had a tremendous efficacy. *Discussion:* The previously reported efficacy of DC even associated with anti-H1 was rare in our patients. In these non responding cases UVBTL01 and tacrolimus could be an efficient alternative. In literature, skin tests are considered "useful" or "always positive" in generalized CADR. We emphasizes that IDT are not "always positive" in such patients, the value of IDT with different IFN in IDR to analyze potential cross-reactions and the importance of delayed IDT reading (mean delay for positive results: 65h).

Does drug hypersensitivity exist in DRESS?

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Oral comunication

Background: It is necessary to determine if there is both virus reactivation and drug sensitization and the value of drug patch tests (PT) and prick tests (pT) in drug reactions with eosinophilia and systemic symptoms (DRESS). *Patients and methods:* Retrospective, bi-center study conducted in patients referred for a plausible DRESS (class 4 or 5) according to Kardaun et al. criteria and having had PT according to European Society of contact dermatitis guidelines, in the 6 months following the disappearance of the DRESS, done with all drugs introduced within the 6 weeks preceding and in the week following the onset of the DRESS. *Results:* Among the 16 included patients (11 F, 5 M, mean age: 51 years), 12/16 had at least 1 positive PT or pT with a suspected drug. Drug classes with positive PT were: betalactams (4 cases), carbamazepine (3 cases) and 1 case each with proton pump's inhibitors, pristinamycine, fluindione, seropram and corticosteroid (CS). A delayed positivity of pT was observed in 3 cases. In some patients treated with many antibiotics, when other tests were negative, IDT were done. They were positive in 2 cases (glycopeptides, betalactams) inducing a rash on the arms in 1 case, quickly vanishing with CS. In 4 patients, without any angry back we observed a co-sensitization to different classes: betalactams with CS or with carbamazepine, fluindione and glycopeptides and PPI both with glycopeptides and aciclovir. Negative investigations were obtained in DRESS occurring with the intake of allopurinol, pristinamycine, anti tuberculosis, carbamazepine or diclofenac. One patient was re-tested 11 years later and her patch tests with carbamazepine and betalactmas remained strongly positive. *Discussion:* Patch tests but also pT with delayed readings can be of value in investigating DRESS as they were positive in 75% of our cases. DRESS is partly due to herpes virus reactivations, drugs can enhance these virus reactivations but a drug sensitization also can occur. A transient non specific reactivity to drugs due to the strong immunostimulation induced by a virus reactivation could be involved, but this cannot explain why PT would remain positive 11 years after the disappearance of the DRESS. We emphasize that a co-sensitization to different chemical classes can occur as observed in 4 cases. The virus reactivation can be involved by a danger-signal in these drug sensitizations or co-sensitizations.

Drug re-challenges in cutaneous adverse drug reactions: information and effectiveness in the long term management of patients

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Poster

Objectives: The long-term negative value of drug skin tests (ST) and provocation tests (PT) is unknown. The aim of this study was to determine if patients diagnosed as non allergic to drugs after allergic investigations have tolerated subsequent treatments with the initial suspected drug and/or a substitute one. *Patients and Method:* By a phone questionnaire, a descriptive, unicentre, retrospective study was carried out. Patients with negative ST and PT between 1999 and April 2009 were questioned by the same investigator, regarding the occurrence and the tolerance of a re-intake of the drug administered with a good tolerance with progressively increased dosages during the PT under hospital surveillance. The reasons why the drug was not taken again were also studied. *Results:* 349 patients (637 RC) were included, 134 drugs were taken again (group A) and 359 were not (group B). We reported 12 reactions (9%) in group A, none of them was severe. In group B, the main reason of the absence of re-intake the drug was the fact that patients didn't need any new course of the tested therapeutic (76.3%) especially with antibiotics or radiocontrast medias. In some other cases the general practitioner (GP) was too fearful in prescribing the drug again. *Discussion:* 91% of the patients with negative RC and a subsequent re-exposure to the suspect drug have a good tolerance of the later treatment. The intolerance after negative skin tests and RC could be due to different mechanisms. Most of the patients reported that these allergic investigations had been useful for them. *Conclusion:* The negative provocation test do not guarantee that a reaction will not recur, for this reason we could think about an improvement of the procedure. In addition, these investigations, well considered by the patients have to be accompanied by clear information of the patient and his GP.

Does the multiple drug hypersensitivity exist?

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Poster

Introduction: A lot of patients report on their multiple allergies to different drugs. Our objective was to propose criteria to define what a multiple sensitization is and to determine if multiple drug hypersensitivity (MDH) exists. *Materials and methods:* From a database with all the patients referred for a cutaneous adverse drug reactions (CADR) since 2000, we selected patients responding to the following criteria of MDH: at least 2 CADR compatible with a mechanism of hypersensitivity occurring with molecules chemically unrelated, occurring more than one month after the first episode of CADR (one year after a DRESS), confirmed by positive skin tests (patch test, prick test, IDT) with a controlled specificity or provocation tests (PT). Exclusion criteria were: CADR related to a pharmacological mechanism (e.g. NSAID intolerance), hematological diseases or HIV seropositivity, chronic wounds (which induced polysensitization to topical drugs) or sensitization to excipients. *Results:* 4/1840 included cases were classified as polysensitized. Case 1: A 76-year-old woman had 2 maculopapular rashs (MPR) and a swelling of the arm due to ibupropfene and pseudoephedrine (positive patch test with pseudoephedrine), domperidone (positive prick test with domperidone) or influenza vaccination (delayed positive reactions on IDT with influenza vaccines). Case 2: A 53 y.o.patient had several episodes of swelling of the face with rfampicin, isoniazid and various macrolides. The IDT with diluted rifampicin and erythromycin were positive (negative controls). Case 3: A 77 y.o. man had a MPR with-ampicillin, an angioedema with tixocortol. Patch tests were positive with many penicillins and classes of corticosteroids. Case 4: A 59 y.o. woman had an anaphylactic shock with an injection of radiocontrast media (RCM). All the tested RCM were positive by prick test or IDT. She also had angioedema with penicillin (positive PT with oroken) and urticaria with cotrimoxazole (IDT resulted in itching and laryngeal discomfort). *Discussion:* The strict criteria described in this work may select patients with MDH. It has to be distinguished from co-sensitization in which the immunological disturbances induced by a first CADR by a "danger signal" facilitating the sensitization to other drugs. Conclusion: The MDH exists but seems rarer than supposed by patients. A genetic predisposition could be explored by studying the cytokine polymorphism in such patients. But its infrequency does not exclude a fortuitous association of 2 CADR in the same patient.

Adverse reactions to macrolides in children

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Poster

Background: Epidemiological studies show that macrolides are amongst the safest antibiotics, and adverse reactions are rare. Especially for children there are only few evaluation studies. The aim of this study is the evaluation of the reactions and natural history of allergy to macrolides in children. *Methods:* Ten children (3.5-12.5 years of age / 6 boys-4 girls) were referred to our Department with a recent, convincing history of hypersensitivity reaction following macrolide administration (7 to Clarithromycin-3 to Erythromycin). 4 children had urticaria, 4 maculopapular rush and 2 experienced an anaphylactic reaction. Skin prick tests with the offending drug and intradermal tests with the offending drug (when available in i.v preperation), or alternatively to another member of the macrolide family were performed. Provocation challenges were performed in seven children (5 to the offending and 2 to alternative drug). *Results:* All but two children (1 positive ID and 1 positive SPT with negative ID) had negative SPT and ID tests. All the children with negative skin tests who were provoked with the offending drug did not react, as well as those provoked with alternative drug. One child received avoidance recommendation due to positive ID test with history of anaphylactic reaction. *Conclusion:* Our results support the notion that adverse reactions to macrolides, -except from being rare, usually the offending drug can be eventually tolerated. Additionally it appears that reactions to macrolides are unlikely to be class allergies, since children with history of reaction to a macrolide, with positive skin test, may tolerate another macrolide drug.

Diagnostic tools in vitro

Indentification of drug-reactive proliferating cells in drug-induced lymphocyte stimulation test

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Poster

Background: Drug-induced lymphocyte stimulation test (DLST) or lymphocyte transformation test (LTT) is a useful tool to determine culprit drug of severe drug eruption such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrosis. Although DLST is widely examined, precise immunological mechanism of this in vitro test is still unclear. *Purpose:* By using flow cytometry (FCM), we tried to identify drug-reactive proliferating cells that correlate to 3H-thymidine incorporation in the conventional DLST. Methods: Freshly isolated peripheral mononuclear cells (PBMCs) from the patients were labeled with CFSE and were cultured with culprit drugs by serial dilutions. On day 6, 3H-thymidine or BrdU was separately added into the culture media. After additional 24-hour culture, cells were harvested and incorporation of 3H-thymidine and BrdU were measured by liquid scintillation counter and FCM, respectively. For FCM analysis, cell surface CD4, CD8 and intracellular BrdU were stained, and CFSE dilution was assessed in the same sample. *Results:* In DLST using FCM, proliferating cells within the last 24-hour culture were detected as CFSElow BrdUhigh population, in correlation with the incorporation of 3H-thymidine by conventional DLST. Proliferating cells were either CD4+ or CD8+, indicating that T cell proliferation is detected in conventional DLST. In particular, CD8+ T cells were dominantly proliferated in the case of SJS. Thus, DLST using FCM can be utilized to study the role of cytotoxic T cells in the severe drug eruptions.

The in vitro platelet toxicity assay (iPTA): a novel diagnostic test for drug hypersensitivity syndrome (DHS)

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Poster

Background/Aim: Drug hypersensitivity syndrome (DHS) is a rare but potentially fatal adverse drug reaction (ADR) which develops in susceptible patients following exposure to certain drugs. The diagnosis of this type of ADRs is quite challenging because of its ill-defined clinical picture and resemblance to other conditions. Currently, the diagnosis of DHS depends on taking good medical history and the expertise of the treating doctor as no reliable and safe diagnostic test is available. In vitro tests including the lymphocyte toxicity assay (LTA) and the lymphocyte transformation test (LTT) are not well characterized and their clinical value is unknown. One of the main drawbacks of such in vitro tests is their complicated procedure which involves isolating peripheral lymphocytes using multiple steps of centrifugation over gradient of synthetic sucrose polymer media (Ficoll TM). The process is time consuming and requires special reagents which may not be available in every lab. The aim of this work was to explore the possibility to using another blood cell type as a surrogate cell model for the in vitro toxicity assay. Such cells would have to be easier to obtain from peripheral blood samples than lymphocytes and respond similarly to in vitro chemical insult. *Methods:* Blood samples were obtained from 10 subjects (5 drug hypersensitive patients and 5 healthy controls) and platelets were isolated by differential centrifugation and incubated with either the culprit drug or its metabolites. The degrees of cell death were then determined and expressed as a percentage of control (vehicle without drug). *Results:* The in vitro platelet toxicity assay (iPTA) was positive in three clinically confirmed hypersensitive patients and in two patients with positive LTA who were never exposed to the culprit drug. On the other hand, it was negative in five health controls. *Conclusion:* While our sample size was not adequate to use comparative statistics and draw a firm conclusion, the obtained results are promising. Here we report the development of a novel diagnostic test for DHS utilizing peripheral blood platelets (PBPs). Isolation of PBPs is quick and simple and does not need special reagents. This simplified test has the potential to be used for routine clinical testing and patient screening for susceptibility to DHS.

The diagnostic value of the lymphocyte toxicity assay (LTA) for drug hypersensitivity syndrome (DHS): A follow-up survey on a cohort of previously tested patients

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Poster

Background/Aim: Drug hypersensitivity syndrome (DHS) is a rare but potentially fatal adverse drug reaction that develops in susceptible patients following exposure to certain drugs. Due to its variable clinical picture and resemblance to other diseases the diagnosis of DHS is challenging. The lymphocyte toxicity assay (LTA) is an in vitro test that has been suggested as a valuable tool in the diagnosis of DHS. However, its predicative value, sensitivity and specificity are still controversial due to lack of a 'gold standard' test to measure it against. The aim of this study was to determine the predictive values of the LTA in diagnosis of DHS based on re-exposure (oral rechallenge) to the culprit drug in a cohort of previously tested patients. *Methods*: One hundred forty seven patients were recruited to participate in this study. These patients had developed hypersensitivity reactions to different drugs and were tested using LTA to confirm their clinical diagnosis. Patients were interviewed by telephone to identify events of re-exposure. *Results:* We identified 26 re-exposure events in 22 patients: 4 were true positives, 17 true negatives, 1 false positive, and 4 false negatives as determined by oral rechallenge. The NPVs of the test in cases of β-lactam antibiotics, sulfonamides and aromatic anticonvulsants were 72.7%, 100% and 80%, respectively. *Conclusion*: The LTA is potentially a valuable diagnostic tool for DHS; however, its NPV vary according to the type of drug tested.

Development of a rapid immunochromatographic test for the detection of high-level serum granulysin in an early stage of SJS and TEN

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Oral communication

Background: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening adverse drug reactions. In the early stage, clinical presentations of SJS/TEN are very similar to those of ordinary types of drug-induced skin reactions (ODSRs); therefore, the diagnosis of SJS/TEN is difficult and the start of treatment is often delayed, resulting in high mortality rates. Recently we reported that serum granulysin level of patients with early stage of SJS/TEN (before mucocutaneous erosion appearance) is higher than that of ODSRs (Abe R, Ann Intern Med 151:514-5, 2009). In this study we developed a rapid immunochromatographic assay for the detection of high-level serum granulysin to diagnosis SJS/TEN. *Materials and methods:* In the immunochromatographic test, a granulysin mAb (G1mAb) was used for conjugation with microparticles. Another granulysin mAb (G2mAb) was immobilized onto a membrane to form a result line. The granulysin in the serum sample specifically bound to the microparticles via G1mAb and comigrated upward until the granulysin was sandwiched with the immobilized G2mAb, revealing a visible result line. Thus, a granulysin-positive specimen (>10 ng/ml) yielded a result line. The entire test procedure was completed in less than 15 min. *Results:* In order to examine the reliability of the testing procedures, we carried out the immunochromatographic assay with 5 serum samples from early stage SJS/TEN patients before mucocutaneous erosion developed and those of ODSRs (n=24). The assay showed a sensitivity of 80% (4 of 5 SJS/TEN patients) and a specificity of 95.8% (23 of 24 ODSRs patients). *Conclusion:* We concluded that the results of the immunochromatographic test are in excellent accordance to early diagnosis for SJS/TEN.

Drug-specific lymphocytes in non-immediate β-lactam reactions in patients with cystic fibrosis

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Poster

Background: Antibiotics are essential for the effective management of patients with cystic fibrosis. Frequent allergic reactions, in particular to β-lactams, have been documented. The aim of this study was to assess the potential role of T lymphocytes in non-immediate β-lactam allergy in patients with cystic fibrosis. *Methods:* A cohort of 45 patients with cystic fibrosis (9 tolerant, 36 allergic) were studied using skin testing (skin prick and intradermal test) and the in vitro lymphocyte transformation test. *Results:* Drug-specific lymphocyte proliferative responses were detected in 19/28 piperacillin-allergic (68%), 3/12 meropenem-allergic (25%) and 1/15 aztreonam-allergic (7%) patients. The sensitivity of the lymphocyte transformation test was 87% with piperacillin allergic patients receiving less than 10 courses of intravenous antibiotic. Ceftazidime-specific lymphocyte responses were not detected. The lymphocyte proliferative response was dose-dependent and results were reproducible. Lymphocytes from tolerant patients with cystic fibrosis and healthy volunteers were not specifically stimulated. Piperacillin-stimulated lymphocytes secreted an array of cytokines including TNFalpha, IL1beta, IL6, IL13, and MIP-1beta. Positive delayed readings following intra-dermal testing were detected in only 4 piperacillin allergic patients. *Conclusion:* These findings show the presence of antigen-specific lymphocytes in blood of drug-allergic patients with cystic fibrosis. This work was conducted in the MRC Centre for Drug Safety Science [grant number G0700654]. The project received funding from the Wellcome Trust [grant number 078598/Z/05/Z)].

The in vitro induction of T cell lines and the use of a stable conjugate between human serum albumin and penicillin amplify the detection of beta lactam-specific memory T cells in patients with ADRs

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Oral communication

Introduction: Beta lactams (bL) are frequently involved in adverse drug reactions (ADR) but the available in vitro diagnostic tests exploring IgE levels or T cell involvement (LTT) exhibit low sensitivity. Thus, additional in vitro tools amplifying the detection of hapten-specific responses would be hopeful. We selected 69 patients with ADR to bL and evaluated serum IgE, LTT assay and hapten-specific short term T cells lines (TCLs) to evaluate the presence of specific T cells at very low frequencies in the circulation. *Methods:* serum IgE to bL were evaluated by ImmunoCAP. LTT was assessed by thimidine incorporation in response to penicillin (pen), ampicillin (amp) or amoxicillin (amox). bL-specific TCLs were assessed for specificity in MHC-dependent and independent conditions. The stable conjugate HSA-pen was tested in LTT in a selected group of patients and normal donors. *Results:* 15/69 (22%) patients exhibited IgE in the circulation towards the culprit drug (6/37 immediate, 9/32 delayed ADR). 12/15 patients also showed IgE to other (on or more) bL. 35/69 (51%) patients exhibited positive LTT to the culprit hapten (19/36 immediate and 16/33 delayed ADR). In addition, 19 LTT+ patients resulted in positivity against additional (one or two) bL (14 and 5, respectively). No specific proliferation towards bL was observed in 30 normal donors. TCLs derived from 37/69 total patients (24 immediate, 13 delayed ADR) were screened for hapten specificity in both MHC-dependent and independent conditions. Comparable proportions of TCLs were specific for pen (46%), amp (49%) or amox (51%) with MHC-dependent response found in 12 (pen), 10 (amp) and 15 (amox) and MHC-independent in 9, 14 and 15, respectively. Only a proportion of TCLs exhibited proliferative response to drugs in both conditions. To note, 15 patients with proliferating TCLs were LTT negative. In a selected group of 17 patients an HSA-pen conjugate was assessed in a proliferative assay. Only 3 patients had a positive LTT to pen but 10 recognized HSA-pen as an antigen. *Conclusions:* LTT represents a valuable diagnostic tool to confirm bL-recognition in both delayed and immediate ADRs. In addition, the induction of TCLs in negative LTT screened in MHC-dependent and independent conditions, allows to enlarge the proportions of patients with confirmed recognition of bL. The use of stable conjugates between human serum proteins and bL may also further increase the sensitivity of proliferative responses.

DRESS syndrome - Circulating drug-specific T cells are detected by ELISPOT assay

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Poster

DRESS is a serious delayed allergic hypersensitivity (DAH) syndrome characterized by multiorgan involvement, lymphocyte activation, eosinophilia, and frequent virus reactivation. Patch testing may be interesting for characterization of the drug responsible for DRESS (1). More recently, ELISPOT assays able to detect drug-specific, cytokine-producing, circulating T cells were developed for diagnosis of DAH expressing as exanthema (2). Here, we report that ELISPOT assay can detect specific T cells in patients with DRESS. *Materiels and methods:* Between 01/2009 and 01/2010, seventeen patients with DRESS (Kardaun score >5) and either negative patch tests (n=6, Group A) or positive patch tests to drugs (n=11, Group B) [amoxicilline (2 cases), imipeneme (1), amikacine (1), acetazolamide (1), fluindione (1), tetrazepam (2), trimethoprim-sulfamethoxazole (1), trimebutine (1), pristinamycine (1) and diltiazem (1)] were included in the study. Characterization of circulating specific T cells was carried out using ELISPOT assay for the detection of IFNγ, IL-17, Granzyme B (GrB) and IL-5 after restimulation of PBMC with the culprit drug and/or a control drug (negative in path tests). *Results:* Elispot assay was positive for 10/11 patients with positive skin tests (Group B) and negative for the 6 patients with negative skin tests (Group A). Therefore, there is a

correlation between the detection of specific T cells by in vivo skin patch tests and ex vivo ELISPOT assay. Drug-specific T cells found in the blood of Group B patients produced both IFNγ, IL- 17 and GrB. The mean number of specific T cells were 64+/-8 (IFNγ), 59 +/- 5 (IL-17), 38 +/-5 (GrB). IL-5-producing cells were not found. *Conclusion:* Our results show that the ELISPOT assay is useful for characterizing the drugspecific T cells in the blood of patients with DRESS and positive patch tests. Since skin tests may induce flares of DRESS, ELISPOT assay could be proposed as an alternative method for the etiological diagnosis of DRESS.

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An experimental biological test to diagnose hypersensitivity reactions to carboplatin

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Poster

Background: Carboplatin is a second generation platinum compound, effective in a lot of cancers. This drug has increasingly been reported to cause hypersensitivity reactions (HSR), mainly in adults. Current data suggest that most of reactions are immunologically mediated and clinical features include symptoms of type I HSR. No biological test is actually available to detect specific IgE in the sera of allergic patients. *Aim:* to evaluate the sensibility and specificity of a new experimental biological test in patients with suspected IgE mediated reactions to carboplatin. The authors present the first results. *Methods:* 3 patients with a high suspition of type I reactions to carboplatin undergone skin tests with an undiluted aliquot from a carboplatin preparation planned for infusion. First, we performed a prick test and, if negative, an intradermal test (0,02 mL); a blood sample of each patient was collected and stored at -20°C. Total serum IgE and specific IgE to carboplatin were determined with the ImmunoCAP system (Phadia AB, Uppsala, Sweden). The carboplatin ImmunoCAP is an experimental prototype, made by immobilization of conjugates of carboplatin with human serum albumin. The limit of quantitation for the sIgE test is 0.1 kUA/l and levels above 0.1 kUA/l indicates sensitization to the specific allergen. *Results:* The first patient, a 65 year-old woman, developed diffuse erythroderma and dyspnoea during the 7th cycle of chemotherapy; the second, a 61 year-old woman , had anaphylactic shock at the 8th administration of carboplatin and the third, a 3 year old child, presented generalized exanthema, disfonia and lips edema during the 6th administration of therapy. In 2 patients intradermal tests were intensely positive. The third patient had negative prick test and didn't undergone intradermal test. Total serum IgE were 191 kU/l, 200 kU/l and 400 kU/l respectively. Also specific IgE antibodies against carboplatin were positive at 0,15 kUA/l, 0,22 kUA/l and 1,6 KU/Al. In patient 3, as Cisplatinum could be the alternative treatment, specific IgE was measured also with high positive results-4.2 kU/l. *Comments:* These preliminary results, with an experimental biological test to diagnose hypersensitivity reactions to carboplatin, demonstrates the relevance of this test to confirm IgE mechanism when studying patients with suspected type I reactions to these compounds. Also, of outmost importance, is the possibility to study the cross reactivity among these agents in order to help in the appropriate therapeutic measure to be taken. More cases have to be studied to determine the sensibility and specificity.

The lymphocyte transformation test is a useful in vitro test in different types of drug induced hypersensitivity reactions

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Poster

Background: We have been using the lymphocyte transformation test (LTT) as an in vitro diagnostic tool for drug hypersensitivity since the early seventies. It is generally accepted that its sensitivity is limited, about 60-70% according to published data, but its high specificity, 85-95% is debated. To determine the clinical reliability of positive LTT results, we looked at the clinical data of patients with positive LTT who were hospitalized for severe drug hypersensitivity reactions over a 2.5-year period at our clinic. *Materials and methods:* 65 patients were hospitalized during this time interval for severe drug induced hypersensitivity reactions. All patients took more than one medication prior to the appearance of the symptoms. LTT was performed after resolution of symptoms and clearance of anti-allergic medications. All patients had positive LTT for only one drug. PBMCs were separated from peripheral blood and cultured in RPMI supplemented with HEPES-buffer and 10% autologous plasma. PHA stimulated cells and cells cultured without drug served as controls. After 72 hours in culture, cell number was determined by MTT assay. Drug concentrations were calculated according to therapeutic blood concentrations. 2.5x of the negative control was considered positive. *Results and conclusion:* Due to the necessity of clinical admittance for these patients, their drug reactions were clinically well defined and their drug related histories were precise. The following drug induced reactions occured: severe anaphylaxis, urticaria, angioedema, maculopapulous drug reaction, erythaema nodosum, fixed drug eruption, erythaema multiforme, Stevens-Johnson syndrome, AGEP (acute generalized eaxnthematous pustulosis) and TEN (toxic epidermal necrolysis). All patients took more than one drug before their hypersensitivity reaction developed and none of them had positive LTT for more than one medication. Of the drugs, antibiotics and NSAIDs were most often implicated, as causative agents. In this set of patients, all positive LTT results were clinically relevant based on the patients' clinical data.

Are lymphocyte transformation test and basotest suitable for immunomodulating drugs? A case report

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Poster

Background: Toxic Epidermal Necrolysis (TEN) is an uncommon life-threatening cutaneous eruption with systemic features which arises primarily among the first eight weeks of medication. In-vitro assessment could be suitable. Infection, vaccination and graft-versus-host disease are additional causes. *Materials and Methods:* A 33 year-old female suffering from Multiple Sclerosis was receiving Beta1a-Interferon from the last 2.5 months, Deflazacort 1 month and Ibuprofen sporadically due to Beta1a-Interferon flu-like side effects. Burning confluent morbilliform erythematous macules beginning at her neckline and thorax spreading to palmoplantar and extremities regions with denudating blisters, malaise, fever, cough and arthromyalgia were becoming patent, while generalized epidermal sloughing, painful stomatitis and mucositis appeared so Neurology Department referred the patient to our Allergy Department. Positive Nikolsky sign, Superficial Punctate Keratitis and Perionyxis were noticeable. Skin Biopsy was performed. Cyclosporine 200 mg/24 hours, Prednisone 50 mg/24 hours on a tapering basis, Zinc Sulfate baths and cessation of culprit medication diminished her symptoms. Allergy study: A Basotest kit and Lymphocyte Transformation Test (LTT) were effectuated in order to enlighten the etiology. Drugs tested were all implicated in the reaction (ibuprofen, interferon and deflazacort) and also Glatiramer Acetate, an immunomodulator which shifts the population of T cells from pro-inflammatory Th1 cells to regulatory Th2 cells, as a suitable treatment instead of Interferon. In vivo tests: Due to reaction severity only alternative drugs were tested (acetaminophen, prednisone). *Results:* Consistent diagnosis of TEN. Skin Biopsy: degeneration of keratinocytes, scarce eosinophils and interphase dermatitis. Inconclusive Basotest and LTT results with Beta1a-Interferon, Glatiramer Acetate, Deflazacort and Ibuprofen. Oral provocation tests to Acetaminophen and prednisone were negative. *Conclusion:* We present a rare case of Toxic Epidermal Necrolysis due to several drugs in which In vitro Tests have been unhelpful in our case to handle this severe and life-threatening event. Owing to the absence of evidence using these recent techniques to assess in-vitro severe drug reactions, further investigation would be useful to clarify its suitability, especially in immunomodulating medication.

Comparison of CD69 measurement, cytokine secretion and LTT in a prospective study

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Poster

Background: The diagnosis of drug-induced delayed type hypersensitivity reactions (DTH) by in vitro methods is an area of intensive research. The in vitro lymphocyte transformation test (LTT) has been reported to have a higher sensitivity than skin tests, but the practicability of LTT is limited. Alternative in vitro tests like the drug-specific CD69 upregulation or drug specific cytokine determination are of great interest, but were not yet compared to LTT. *Aim:* To determine the cut-off for positivity and the sensitivity/specificity of three drug specific assays, we compared CD69 upregulation and cytokines secretion with the LTT. *Methods:* In this prospective study 182 CD69 tests (72h PBMC culture, measurement of CD69) and LTTs (7 days PBMC culture, measurement of proliferation) were performed in 97 patients with 68 different drugs. The cell culture supernatants (72h, 2-4 drugs/subject) were analyzed in 18 patients and 14 controls for 10 cytokines (INF-γ, IL-1b, IL-2, IL-5, IL-4, IL-8, IL-10, IL-12p70, IL-13, TNF-α). Patients were classified according to previously established criteria into 4 clinical probability groups (drug allergy definitive, probable, doubtful or highly unlikely). Cut-off for positivity was calculated based on a group of 20 (CD69) and 14 (cytokines) healthy controls exposed to the drugs and without adverse reactions. *Results:* In all patients with an estimated probability of a drug allergy of >10% (which included also non drug allergic individuals), the sensitivity and specificity of the CD69 test was 16.7% and 97.8% and for LTT 11.6% and 96.8%, respectively, whereas the combination of both tests yielded a sensitivity of 20% and 26.7% respectively. The specificities in the cytokine secretion assay were very variable depending upon the cytokine measured (0-28% for individual cytokine), whereby IL-5 (28%), IL-2 (21%), and IL-12p70 (17%) showed the highest values. With the combination of IFN-γ, IL-2, IL-5, IL-8, and IL-12 the sensitivity could be increased to 48% with a specificity of 82%. *Conclusions:* The sensitivity of CD69 test and LTT is lower than previously reported, which might be due to difficulties in classifying patients in a prospective study as truly drug allergic. Cytokines assays were positive almost exclusively if the prior LTT or CD69 assay was positive, but allowed to detect additional reactions. Therefore, a stepwise approach is advisable: first LTT or CD69 determination, followed, if negative, by combined cytokine assays.

Severity of Aspirin/NSAID hypersensitivity syndrome with and without a history of paracetamol sensitivity

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Poster

Background: Aspirin/NSAID hypersensitivity syndrome is an immediate hypersensitivity reaction in response to Aspirin and/or NSAID consumption. Some patients reported to have similar symptoms with paracetamol as well. As the proposed mechanism of this syndrome is related to imbalanced leukotriene pathway, patients who develop similar reactions to paracetamol, a weak cyclooxygenase inhibitor, theoretically may have severe clinical manifestations. *Materials and Methods:* We comparatively studied 33 aspirin/NSAID hypersensitivity patients, 16 of them (12 F/4M), 43.9 years old (range 16-70), were

paracetamol-tolerant (PT) and 17 of them (17F), 44.1 years old (range 26-67), also had a history of immediate reactions to paracetamol (paracetamol-sensitive, PS). Nasal provocation test (NPT) with 16 mg of aspirin was performed in all 33 cases and basophil activation test (BAT) with 1.25 mg/mg of lysine-aspirin was performed in 14 PT and 15 PS patients. Nasal symptom score at least 5 out of 15 and/or 25% decrease of total nasal volume from baseline measured by acoustic rhinometry was defined as a positive NPT. The increase of percent activated basophils (CD203c+ve) at least 5% from non-stimulated control with the stimulation index > 2 was considered a positive BAT. *Results:* Clinical presentations after drug exposure were analysed in both groups. In PT group, 6 patients developed respiratory symptoms (3 naso-occular, and 3 naso-occular with asthma) and 10 patients developed mucocutaneous symptoms (6 urticaria, 2 angioedema, and 2 urticaria/angioedema). In PS group, 11 patients developed respiratory symptoms (2 asthma, 4 naso-occular, and 5 naso-occular with asthma) and 6 patients developed mucocutaneous symptoms (1 urticaria, 2 angioedema, and 3 urticaria/angioedema). Symptom onset after drug exposure was significant longer in PT group than that in PS group (85 minutes and 39 minutes in PT and PS, respectively, P value <0.05). Multiple NSAIDs sensitivity were reported in 6/16 patient in PT group and in 8/17 patients in PS group. Nasal volume reduction after lysine-aspirin NPT were 36.3% in PT (NPT+ve 10/15 cases) and 28.6% in PS (NPT+ve 11/16 cases). Percentages of activated basophils with BAT were 18.8% in PT (5/14 BAT+ve) and and 15.2% in PS (7/15 BAT+ve), respectively. The results of nasal provocation tests and basophil activation tests were not statistically different (P value > 0.05). *Conclusion:* Patients with aspirin/NSAID hypersensitivity syndrome with a history of paracetamol intolerance developed shorter symptom onset after drug exposure compared to paracetamol-tolerant group. Contrary to the original believe, however, patients with a history of paracetamol sensitivity did not demonstrate more severity parameters compared to paracetamol tolerant group.

IgE - Anaphylaxis

Anaphylaxis to omeprazole

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Poster

Introduction: Proton pump inhibitors (PPI) are widely used and are generally well tolerated, with a low incidence of adverse reactions. Although immediate-type hypersensitivity reactions induced by omeprazole and other PPI are rare, several anaphylactic reactions have been reported. Case report: A 37-year-old woman, with no personal or family history of atopic disease, was referred to our unit after an episode of facial, upper and lower members urticaria, nausea, vomit and dyspnea with wheezing, 10 hours after oral ibuprofen (600 mg) and 20 minutes (min) after omeprazole 20 mg (prescribed for an acute pharyngitis). She denied any other drug sensitivity or any other concomitant therapy. She had previously taken omeprazole with good tolerance and she didn't remember if she had already taken ibuprofen. Skin prick tests (SPT) to aeroallergens were negative. SPT (4 mg/ml) and intradermal tests (IDT) (0,04 mg/ml) with omeprazole were positive. Basophil activation test with omeprazole was negative. After a patient´s written informed consent we performed a challenge test with omeprazole and the patient experienced anaphylaxis (urticaria, cough, nausea and hypotension) 5 min. after 10 mg of omeprazole. The clinical picture resolved 60 min. after iv administration of clemastine, hydrocortisone, methylprednisolone and ranitidine. SPT with pantoprazole (4 mg/ml) and with lansoprazole (30 mg/ml) were negative. IDT with pantoprazole was positive (0,04 mg/ml). IDT with lansoprazole were not performed (not available). Challenge with lansoprazole was not performed yet. Total IgE was 36,9 Ul/ml. The patient self-administered oral ibuprofen at home with no reaction. *Discussion:* We present a patient who experienced an anaphylaxis episode after oral intake of ibuprofen and omeprazole (prescribed for an acute pharyngitis). The clinical findings and the positive SPT to omeprazole suggest that an IgE-mediated mechanism was involved in the reaction to omeprazole. We excluded hypersensitivity to ibuprofen because the patient self-adminestered it at home with no reaction. Although PPI are extensively used and generally well tolerated, anaphylactic reactions can sometimes be observed. The positivity of IDT with pantoprazol suggests cross-reactivity between PPI, as other authors have reported. It is recommended an allergologic study, including skin and controlled challenge tests, before offering other PPI as a safe alternative.

IgE-mediated Diclofenac allergy: induction mechanism and improvement of diagnosis

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Oral communication

Background: In a 7-years-retrospective analysis of our outpatient clinic population with drug hypersensitivity reactions to non-steroidal anti-inflammatory drugs (NSAID), diclofenac was found to be the most common culprit for severe anaphylactic reactions, suggestive for an IgE-mediated mechanism. This data prompted us to evaluate the mechanisms of diclofenac hypersensitivity more precisely.There are two major questions standing: A) How can sensitization and IgE formation occur against this hapten and B) why is drug allergy diagnosis inconsistent? *Objective:* Oral diclofenac largely binds to albumin in stomach juice thereby acquiring the features of a complete antigen. In analogy to the development of food allergies, we aimed to investigate whether co-medicated anti-ulcer drugs could explain the underlying pathomechanism for IgE-mediated drug sensitization. Second, diagnosis is today based on intradermal tests with monovalent diclofenac, not considering that IgE crosslinking is required for mast cell activation. We thus proposed that a multivalent diclofenac conjugate could improve drug allergy diagnosis. *Methods:* A) Diclofenac was orally applied to BALB/c mice with or without gastric acid suppression, alone or coupled to mouse serum albumin. Sera were tested in ELISA and

rat basophilic leukemia test, and mice subjected skin tests. B) In two of our patients with generalized urticaria to diclofenac and two control persons, we performed skin prick tests (SPT) and ELISA for IgE antibodies using diclofenac coupled to keyhole limpet hemocyanin (KLH), uncoupled KLH or diclofenac. *Results:* A) Only mice receiving albumin-coupled diclofenac under gastric acid suppression developed anti-diclofenac IgG1 and IgE in a dose-dependent manner. The induced antibodies triggered mast cell degranulation and positive skin tests. B) IgE antibodies in patients' sera towards diclofenac could be detected in ELISA coated with the diclofenac-KLH conjugate. Further, the diclofenac-KLH conjugate elicited wheal and flare reactions, whereas KLH alone or diclofenac (even at a 100 times higher concentration) did not elicit skin prick reactivities. *Conclusion:* A) Gastric acid suppression is suggested a causative mechanism in the induction of IgE-mediated diclofenac allergy. B) Testing with diclofenac-KLH conjugate improved diagnosis of IgE-mediated diclofenac allergy. *Acknowledgements:* This work was supported by FWF project L467-B05.

Anaphylaxis to drugs – data from the anaphylaxis register of German-speaking countries

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Poster

Background: In the anaphylaxis register NORA of the german-speaking countries, severe anaphylactic reactions with respiratory and/or cardiovascular symptoms are documented since 2006. To date 78 hospitals and specialized centres are participating. Herein we report on 221 severe immediate reactions to drugs which were registered between 07/2006-02/2009. *Patients and Results:* Among 1165 registered anaphylactic reactions, 221 (18.9%) were attributed to drugs. In the overall cohort drugs are the third important causative agents after insect venoms (49.4%) and food (23.3%). An age related analysis shows that in adults (≥18 yrs), drugs are the second most frequent elicitor (n=204; 21.1%) after insects (n=529; 54.6%). Among children and adolescents (n=197) drugs (n=17; 8.6%) are in third place (foods: n=115; 58.4%, insects: n=47; 23.9%). 61.1% (n=135/221) of all patients developed skin-related symptoms, only 15.8% (n=35/221) gastrointestinal symptoms. Respiratory symptoms were present in 116/221 patients (52.5%) and cardiovascular/vigilance-related symptoms in 55.2% (n=122/221). No reaction was fatal. In 67/221 cases (30.3%), the reaction documented in the database was not the first event. 47% (n=105/221) of the reactions occurred at a physician's office or in hospital, 27.6% (n=61/221) at home. Apart from a thorough history (n=217/221, 98.2%), skin tests were most commonly used as diagnostic means (n=164/221; 74.2%), followed by measurement of mast cell tryptase (n=117/221, 53%), specific IgE (n=96/221, 43.4%) and provocation tests (n=63/221; 28.5%). The most frequently incriminated drug-group were NSAID (n=82/221, 37.1%), followed by antibiotics with 49/221 reactions (22.17%), local anaesthetics (n=25/221; 11.3%) and others (n=64/221; 29%). Potentially aggravating conditions or concomitant diseases, such as atopic or cardiac diseases, chronic urticaria, malignancies etc. were present in 106/221 patients (48%). 2 patients suffered from mastocytosis (0.9%). *Conclusion:* The data provides useful information on elicitors and concomitant factors in drug-induced anaphylaxis. It also provides insight into the diagnostic means taken to confirm a clinical diagnosis and to identify the causative drug. A relatively high rate of recurrent reactions indicates the need of better patient information and instruction.

Leukotriene involvement in immediate reactions occurring during anaesthesia

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Poster

Background: Immediate reactions occurring during anaesthesia are usually classified as allergic hypersensitivity (AH) where skin tests and mast cell tryptase are positive, and non-allergic hypersensitivity where negative results are obtained. Leukotriene is a potent mediator synthesized and excreted as a result of IgE-antigen binding, which could play a role in acute allergic events. *Materials and methods:* Plasma leukotriene C4, D4, E4 concentrations were measured by ELISA test (Cell Com) in serial samples obtained from 21 uneventfully anaesthetized controls, after IRB approval and written informed consent. The values were compared retrospectively with those of 27 patients who reacted during anaesthesia, 19 who had increased tryptase and positive skin tests (AH group) to the administered neuromuscular blocking agent (NMBA), and 8 who had normal tryptase and negative skin tests to all administered drugs and latex (N-AH group). *Results:* In the control group, the mean leukotriene concentrations were 0.96 +/- 0.52 µg/L before induction of anaesthesia; 0.99 +/- 0.42; 0.79 +/- 0.28; 0.89 +/- 0.33 at respectively 30 min; 6h; 24 h thereafter. The values of the AH group were: 28.9 +/- 25.6 µg/L at 30 min, and 20.2 +/- 13.7 at 24 h, significantly higher than the control group, whereas the values of the N-AH group were 8.2 +/- 5.5 µg/L at 30 min, and 9.5 +/- 8.5 at 24 h, significantly higher than the control group and smaller than the AH group. *Conclusion:* Leukotriene concentrations were stable during the anaesthetic procedure and surgery in non-reacting controls. The values were considerably increased over 24 hours in patients with proved allergic hypersensitivity reactions, and moderately increased in patients with non-allergic hypersensitivity events. The results suggest that leukotriene is involved in per-anaesthetic reactions through mast-cell mediated as well as non-mast cell mediated mechanisms, opening a new field of investigations for the 40% of reacting patients who have negative allergological tests.

Immediate selective reactions to NSAIDs: clinical and immunological characteristics

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Poster

Objectives: NSAIDs are the most frequent medicaments involved in hypersensitivity drug reactions. In addition to cross-intolerance, se-

lective IgE or T-cell mediated reactions exist. The aim was to estimate the number of immediate selective responders out of a group evaluated with confirmed NSAIDS hypersensitivity. The clinical characteristics and drugs involved are analyzed. *Methods:* A large group of subjects reporting reactions to NSAIDs was evaluated. Skin prick (SP) and intradermal (ID), basophil activation tests (BAT) with pyrazolones, total and specific IgE to aeroallergens and oral provocation tests with NSAIDs were performed. *Results:* From 752 cases diagnosed, 20% had selective hypersensitivity reactions being the 57% immediate reactions. In this group, 70% were female. No differences in total and specific IgE were found compared to a control group. Metamizol was involved in 43%, followed by ibuprofen (17%), diclofenac (15%) and ASA (9%). Anaphylaxis occurred in 57%. No patients had exclusively respiratory symptoms. Skin tests were performed in 37 patients, being positive in 18,18%, all of them to metamizol (8 positive in PT and ID and 2 positive in ID). BAT to pyrazolones was positive in 54,34%. A total of 89 challenges were carried out in order to confirm tolerance to other non chemically related compounds. *Discussion:* Pyrazolones are the most frequent NSAIDs implicated in immediate selective reactions, being anaphylaxis the most frequent clinical manifestation. The sensitivity of skin tests and BAT is low; therefore clinic history and confirmation of tolerance to others NSAIDs by challenges can be needed to achieve the diagnosis.

Sensitization to ethylene oxide in patients with suspected allergic reactions during anaesthesia

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Poster

Background: Ethylene oxide (ETO) is a highly reactive gas widely used for sterilization of medical devices (catheters, infusion sets, surgical drapes etc). ETO is toxic and can cause allergic sensitization. Myelomeningocele patients and haemodialysis patients appear to be high-risk groups. At the Danish Anaesthesia Allergy Centre (DAAC), patients with allergic reactions during anaesthesia are investigated. The aim of this study was to establish the frequency of IgE sensitization to ETO in this population. *Material and methods:* Serum from all patients investigated at DAAC in the period May 2004-June 2009 (n=201) were analyzed for IgE to ETO (ImmunoCAP®, Phadia AB, Uppsala, Sweden). *Results:* Three out of 201 patients had positive tests (1.5%). All three had had multiple operations, and two belonged to high-risk groups (one with spina bifidus and one on haemodialysis). All three patients had another verified allergen: latex, cefuroxime and hydroxyethyl starch (a blood plasma substitute). *Conclusion:* IgE sensitization to ETO in patients with allergic reactions during anaesthesia is existing but rather rare. The clinical relevance of sensitization to ETO remains to be confirmed since all our three patients were allergic to an additional allergen to which they had been exposed - and no confirmational challenge with ETO was performed. However, it is likely that high level exposures to ETO can cause anaphylactic reactions in sensitized patients. Due to the widespread use of ETO, we recommend that investigation of patients with allergic reactions in connection with surgery and anaesthesia include ETO. Specific IgE levels might decrease considerately within few months on lack of exposure, which emphasizes the need for development of additional tests like histamine release test, skin test or provocation test with ETO to support the allergy diagnosis.

IgE-mediated allergy to Group A betalactams: tolerability of cefpodoxime and ceftriaxone

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Poster

Background: IgE-mediated allergy to group A beta-lactams is frequent and often leads to contraindication of all betalactam antibiotics in allergic patients. However, analysis of the chemical structures of betalactams indicates major differences in the reactivities of penicillins and cephalosporins (JA Chemelle, personal data) suggesting that peni A reactive patients may not be ractive, and therefore could tolerate, third generation (3G) cephalosporins. *Objective:* The objective of the study was to analyze cross reactivities between group A penicillins and other betalactams in peni A allergic patients and, based on these findings, to propose alternative drugs in these patients. *Methods:* Between 2003 to 2007, 820 patients with immediate hypersensitivity to group A betalactams received skin tests (Prick test and Intra Dermal Test) and biological tests (specific IgE and /or basophil activation test) to the offending drug. Skin tests with a battery of 17 betalactams and challenge test with negative drugs were carried out in allergic patients. *Results:* A total of 820 patients presenting with immediate hypersensitivity to group A beta-lactams were tested. Among these, allergic assessment confirmed IgE mediated hypersensitivity in fourty patients (36 amoxicillin, 1 cloxacillin and 3 bacampicillin). Crossreactivity with other betalactams was found in most of the 40 patients with a mean number of 4 cross-reactive molecules per patient. When only amoxicillin-allergic patents were considered, 41.1% (14/34) of the patients were found to have cross-reactivity with one or more penicillins, 8.8% (3/34) to cephalosporin and 32.3% (11/34) to penicillin(s) and cephalosporin(s), while 17.6% (6/34) tested positive for amoxicillin only. Interestingly in all cases no cross reactivity was found between amoxicillin and the 3G cefalosporins ceftriaxone and cefpodoxime. Considering bacampicillin and cloxacillin allergic patients, similar resuls were observed. According to this, cefpodoxime or ceftriaxone were reintroduced in 34/40 patients without any adverse immediate or delayed reaction. *Conclusion:* Our study: 1) emphasize the usefulness of allergogical investigations in all peniA hypersensitive patients; 2) confirms the high cross reactivity rate between Group A penicillin and other betalactams; 3) indicate that it is not justified to contra-indicate all betalactams in peni A allergic patients; 4) shows that it is possible to use 3G cefalosporine, especially cefpodoxime and ceftriaxone in peni A allergic patients.

Basophils

Basophil responsiveness and clinical picture of acetylsalicylic acid intolerance

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Poster

Background: Exposure to acetylsalicylic acid (ASA) may exacerbate respiratory or skin diseases or induce anaphylactic reactions in apparently healthy individuals. These adverse reactions are believed to be due to hypersensitivity to pharmacological actions of the drug. However, the cellular basis for those reactions is poorly understood and therefore we wanted to evaluate specific responsiveness of basophils to ASA in correlation with clinical picture. *Materials and Methods:* We performed a prospective single-blind study in 59 subjects involved in clinical evaluation and/or ASA provocation testing. Whole blood basophils were stained with anti-CD63/CD123/ HLA-DR mAbs (BD) after stimulation with 0.25 mg/ml or 1 mg/ml of ASA concentrations. *Results:* We found 40 ASA tolerant (13 asthma/rhinitis) and 19 ASA intolerant (9 asthma/rhinitis) subjects. Intolerant subjects showed significantly higher overall basophil responsiveness to ASA in comparison to tolerant subjects ($P<0.01$), which was concentration dependent in both groups ($P<0.001$). The ratio between the response at 1 mg/ml of ASA and the baseline (activation index) was analyzed according to the clinical picture. We demonstrated that the activation index was significantly higher in intolerant subjects without chronic conditions ($P=0.008$), but not in intolerant subjects with asthma/rhinitis ($P=0.14$), in comparison to matched tolerant subgroups. The ROC calculations showed that the optimal threshold activation index is more then 2.18. In that case the sensitivity added up to 80% and the specificity up to 83% in the subgroup without chronic conditions. In asthma/rhinitis subgroup the sensitivity was 78% and specificity only 50%. *Conclusion:* Our study demonstrated that there is a significantly higher basophil in vitro response to ASA in intolerant then in tolerant subjects. ROC curve analyze suggest that this measurement might have unambiguous diagnostic value only in subjects without chronic conditions such as asthma and/or rhinitis.

Open questions for diagnosis of multiple NSAIDs hypersensitivity by basophil activation

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Poster

Background: Drug hypersensitivity reactions against NSAIDs occur frequently and can cause severe anaphylaxis. Mechanistically multiple NSAIDs hypersensitivity is considered nonimmune-mediated and may result from nonselective coinhibition of constitutively expressed cyclooxygenase 1. NSAIDs have been reported repeatedly to function as stimulators in basophil activation test (BAT) and cellular antigen stimulation test. Stimulation time and dosing represent important parameters for the cells' vitality and reactivity in these assays. Therefore, different assay conditions were investigated for a panel of NSAIDs in regard to cell vitality in parallel to BAT. *Materials and methods:* Blood samples of NSAIDs-tolerant donors were analyzed for basophil activation at various concentrations of diclofenac, ibuprofen, mefenaminic acid, and acetylsalicylic acid. The impact of stimulation time on blood cell vitality was assessed by gating blood cells with low forward scatter. In parallel, the cell population representing lymphocytes according to forward and side scatter characteristic was assayed for apoptosis and necrosis using annexin V binding and 7-aminoactinomycin staining. *Results:* Effective concentrations of NSAIDs under current stimulation conditions of basophil activation test were determined. Dose-dependent basophil activation by diclofenac was observed in multiple NSAIDs-hypersensitive patients and tolerant controls. Optimal stimulation times for significant upregulation of degranulation markers CD63 and CD203c were defined for both groups. Onset of apoptosis under current stimulation conditions was controlled by analysis of cell death markers and specific gating procedures. Comparative staining protocols with isotype controls were used to exclude unspecific effects. *Conclusions:* Several assay parameters including effective concentration of stimulating agent and stimulation time evolved to be crucial for BAT. NSAID-mediated basophil activation was shown to be independent of surface alterations due to early cell death or necrosis. Exact cut-off values need to be defined under well-controlled conditions for diagnosis of multiple NSAIDs hypersensitivity. This work was supported by grant P18820-B13 of the Austrian Science Fund (FWF).

Developing a passive sensitization procedure for basophils to diagnose IgE-mediated drug hypersensitivity

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Poster

Introduction: The basophil activation test (BAT) is quite well established for protein allergens. It is normally done with the patients basophils and requires fresh patients cells and rapid processing. It would be a particularly interesting test for drug allergy, as only very few drug specific in vitro IgE assays exist and as BAT seems to be a highly relevant read out system. *Aim of the study:* To set up a sensitization protocol to test IgE-mediated drug hypersensitivity. *Methods:* PMBCs of healthy basophil donors are incubated with lactic acid (pH = 3.9) to remove surface bound IgE. Subsequently, the "stripped" basophils are incubated with patient serum, presumably containing drug specific IgE, which binds with high affinity to FcεRI. Afterwards, the basophils are stimulated with serial dilutions of appropriate drugs and the degree of activation can be measured by flow cytometry by using a two-color strategy (anti-CCR3-APC/anti-CD63-PE). *Results:* We were able to establish a well functional procedure for protein allergens (hymenoptera venom) and for some drug allergens (carboxymethylcellulose, cephalosporines, chlorhexidine). But BAT was reproducibly false negative with pas-

sive sensitization assays using serum of patients with well documented IgE-mediated amoxicillin allergy. *Conclusion:* Using defined donor basophils for passive sensitizations with serum would have several advantages over direct BAT assays, as it would facilitate diagnosis, as patient's sera is easy to store and as it would provide a direct proof of an IgE-mediated hypersensitivity reaction. However, a better understanding of how small drugs are actually able to cross-link cell surface bound IgE seems to be a prerequisite to improve this assay and to widen its application.

Usefulness of basophil activation test in the management of immediate-type hypersensitivity reactions to quinolones

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Poster

Background: Quinolones are synthetic antibiotics with a broad activity. These molecules are known to have a good safety profile; however, some adverse effects have been reported including immediate hypersensitivity-type reactions (IHR). The diagnosis procedure mainly consists of an evocative clinical history and positive skin test. The purpose of our study was to assess the usefulness of basophil activation test (BAT) in this diagnosis approach. *Methods:* Population: 28 patients who presented an IHR less than an hour after quinolone administration were included in our study. Skin tests: Skin prick tests were done in a first step. Intradermal tests at low concentrations (10-3 and 10-2 dilutions of the prick test solution) were performed if the prick tests were negative. Basophil activation tests: The BATs were performed by measuring CD203c expression in the basophil population using flow cytometry. The positivity threshold was defined on the negative control without any drug. All the results are expressed as percentage of CD203c-positive basophils among the total basophil population. Results were considered positive when at least two sequential drug dilutions induced more than 10% CD203c-positive basophils. *Results:* Among the 28 patients with an IHR, 15 presented a negative BAT to the suspected quinolone. A challenge test was performed in 14 of these 15 patients. All these reintroductions were successful, assessing thus the non-allergic origin of the reaction in these 14 patients. It is to be mentioned that 2 of these 14 patients had positive skin tests. Among the other 13 patients, 1 has an un-interpretable BAT, and 12 were positive for the suspected quinolone. For these 12 patients, with a high probability of immediate allergy to quinolone (10/12 positive skin tests), the culprit drug was not reintroduced. However, BATs were performed using an other molecule of the quinolone family. A quinolone giving a negative BAT was re-administrated to 4 patients: it was successful in 3 of them and in the fourth an urticarial reaction after the second dose challenge (1/100 of the therapeutic dose) was observed. *Conclusion:* Our study clearly highlights the excellent negative predictive value of BAT in the management of patients with a clinical evocative history of IHR to quinolones. Preliminary results also show that BAT is helpful to select a safe non cross-reactive molecule of the quinolone family, avoiding thus the total exclusion of this important class of antibiotics.

Impact of CAST in the diagnosis of drug hypersensitivity and insect venom allergy

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Poster

B*ackground:* The diagnosis of allergic reactions due to drugs and insect venoms is challenging in many cases. History given by the patient is frequently imprecise, the clinical picture unspecific, skin tests and in vitro tests not always satisfying, and provocations potentially hazardous and thus ethically problematic. The Cellular antigen stimulation test "CAST" represents a new tool in the in vitro diagnosis of immediate type allergic or pseudoallergic reactions. It is based on the stimulation of leucocytes and subsequent determination of the sulfoleukotrienes produced. The present study investigated the correlation of CAST with routine diagnostic parameters. *Material and methods:* In a retrospective study, 92 patients with suspected immediate type reactions to either antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs) including acetylsalicylic acid, or hymenoptera venom, in whom a CAST had been performed in addition to the routine test panel, were investigated. Additionally 12 control patients with clearly positive or negative diagnoses were included. *Results:* Over all (n=92), CAST confirmed a positive skin test, with a sensitivity of 91% and a negative predictive value of 94%. Specifity, however, was low with 51%, and a positive predictive value of 40%. For antibiotics (n=54), a positive CAST correlated with all positive routine test parameters (history, positive specific IgE, skin tests) with a sensitivity of 88-100%, but again with a low specifity of 28-39%. For hymenoptera venoms (n=23), we found an excellent correlation with skin test, whereas history and specific IgE were less predicitive. Only 12 patients with immediate type reactions to NSAR were investigated. Here, both sensitivity and specifity with history were low. In 12 control patients, CAST was found to show an excellent sensitivity and specifity of 100%. *Conclusion:* In patients with a positive skin test, CAST is also mostly positive. Thus, it does not provide essential additional information for diagnosis. In all other situations, where the outcome of CAST cannot be predicted, it might present as a useful tool in the diagnosis of drug and hymenoptera venom allergy. At this stage, however, CAST cannot be recommended as a routine parameter but rather an additional information in individual situations.

A new basophil activation test using CD63 and CCR3 in allergy to antibiotics

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Poster

Background: Flow cytometric basophil activation tests have been developed as cellular tests for in vitro diagnosis of IgE-mediated reac-

tions. Different markers and techniques have been used after stimulation with various allergens. It was the aim of the present study to compare an established basophil activation test (Flow-CAST®) with a newly developed basophil activation protocol using CD63 and CCR3 (Flow2 CAST®) in patients with type-I-allergy to antibiotics. *Materials and methods:* 24 patients with a history of type-I-allergy to antibiotics were examined. A careful allergy history was taken, and skin tests and determination of specific IgE-antibodies were performed. The two different basophil activation tests (BAT) using CD63 expression but different protocols were done after stimulation with different concentrations of antibiotics. 15 healthy subjects without history of antibiotic allergy were studied as controls. *Results:* The Flow2 CAST showed a higher sensitivity than the Flow-CAST (55% vs. 53%) with regard to patients' history. Specificity was 80% both for the Flow2 CAST and for the Flow-CAST with regard to controls with negative history and negative RAST. *Conclusion:* These results show the value of two different basophil activation tests as cellular tests in the in vitro diagnosis of patients with antibiotics allergy with equal specificity and a slightly higher sensitivity for the Flow2 CAST.

Basophil activation test in beta-lactam allergy: evaluation of the combination of the activation markers CD63 and IL-3

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Poster

Background: In the recent years, the basophil activation test (BAT) has been proved to be a complementary tool for the study of beta-lactams CD63 and CD203c are the most frequent activation markers used for the BAT. They have rarely been used in combination of Interleukine-3 (IL-3), that is supposed to prime the basophils leading to maximized activation of CD63, but also leads to CD203c. The aim of our study is to evaluate the pros and cons of a new basophil activation protocol using CCR3, CD63 and CD203c, in patients with beta-lactam hypersensitivity. *Materials and methods:* 11 patients with immediate hypersensitivity to beta-lactams (documented by positive skin tests and/or specific IgE) and non-atopic) were tested with BAT using a modified version of the Bühlmann Flow2 CAST®. We used two different concentrations a stimulation buffer with and without IL-3, and the activation markers, CD63 and CD203c, in two different combinations: 1) CD203c-PE-DY647, measuring the activation in two different fluorescence channels; 2) CD63-PerCP / CD203-PE-DY647 one fluorescence channel. Results and conclusion: 11 patients were involved in the study, 10 of them were evaluated, one of them was a non responder. The IL-3 leads intensity for the CD203c marker, but this has not a negative effect on the differentiation between allergic and non-allergic not a negative effect on the activation of CD203c alone, and improves the sensitivity of the combination of CD63-CD203c, CD63 mean net activation (5.8% -> 13.0%), and helping a better differentiation between negative and positive results. found in the combination of both markers in the presence of IL-3. The specificity is 90 to 100% for CD63 and CD203c alone, The specificity is slightly reduced in the combination of both markers (83%) and the cut-off is also higher (net cut-off CD63: In our experience, the combination of CD63-CD203c, in presence of IL-3, improves the sensitivity of the BAT in beta-lactam with the sensitivity of the BAT with the single markers. A larger number of patients is necessary to confirm our results.

Diagnosis of amoxicillin/clavulanic acid allergy using basophil activation test CD203c

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Poster

Introduction: Clavulanic acid is a potent inhibitor of beta-lactamase that is increasingly prescribed in association with amoxicillin. Skin test sensibility for betalactams has been widely reported to be of very good value. Nevertheless, Skin Tests Sensibility in amoxicillin/clavulanic acid (AX/CA) anaphylaxis is not well known. We report two cases of AX/CA severe anaphylactic reaction with negative skin tests (prick, intradermal test (IDT)) and specific immunoglobulin (IgE) but positive basophil activation test (BAT). *Cases reports:* In March 2009, a 67 years old patient presented grade IV Ring and Messner anaphylaxis, 30 minutes after first intravenous intake of AX/CA rapidly reversed by epinephrine and cardiopulmonary resuscitation. In June, we performed AX/CA skin tests (20 mg/ml: prick and IDT 10-3, 10-2) and Specific IgE which were negative. BAT analyzes, by flow cytometry overexpression of CD203c after AX/CA re-stimulation revealed significant positive responses with AX/CA (31%, negative control 3%) and amoxicillin (45%, neg 3%). In July, IgE and new serie of skin tests were negative with AX/CA. The BAT was not interpretable (lack of negative control). In February 2010, skin tests with AX/CA were negative, but the basophil activation test was positive (13%) with AX/CA. According to the BAT and severe reaction we concluded to IgE mediated allergy to AX/CA. Five years ago, A 40 years old woman experienced grade II Ring and Messner anaphylaxis (urticaria and angioedema), 40 minutes after first oral intake of AX/CA resolved after corticosteroids injection. IgE and skin tests (prick, IDT) realized three times (July, November and December 2009) were negative with AX/CA. BAT after stimulation with AX/CA revealed significant positive responses two times (july and December 2009) at 15, 31% CD203c respectively and negative for amoxicillin with negative control of 2%. Because of relevant clinical symptoms we concluded to Allergic IgE mediated reaction to AX/CA. *Discussion-Conclusion:* BAT has often been described to be specific and sensitive with amoxicillin. Recently BAT interest has been reported in few cases of Acid clavulanic anaphylaxis with negative skin tests (1). Nevertheless our work was, the first to our knowledge, using CD203c as basophil activation marker in AX/CA anaphylaxis. Our present study confirms the BAT interest in AX/CA allergy with negative skin test. Indeed BAT could be the only positive assessment even in case of grade IV reaction as in our cases and could thus avoid potentially dangerous challenge test.

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Flowcytometric BAT could help in the in vitro diagnosis of multiple allergy drug syndrome

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Poster

Background: Multiple-drug allergy syndrome refers to a condition characterized by an increased propensity to react to immunologically and chemically unrelated drugs. Antibacterial drugs and nonsteroidal anti-inflammatory drugs are the most frequent causes of MDAS but other drug classes may be involved in this condition as well. *Material and methods:* we selected 12 patients (3 male/9 female) mean age 46 years with clinical history of multiple acute orticaria episodes with or without angioedema after the administration of various unrelated drugs. Reported offending drugs were: betalactams (n=10/12), nonsteroidal anti-inflammatory drugs (n = 11/12), lidocaine (n = 4/12), cephalosporin (n = 4/12). We performed flowcytometric BAT (Flow CAST, Bülhlman Laboratories, Allschwill, Switzerland) to penicillin, ampicillin, amoxicillin, PPL, MDM, aspirin, ibuprofen, paracetamol, diclofenac, lidocaine and cephalosporin. *Results:* We obtained positive results with penicillin (n. patients = 4/10), ampicillin (n. patient = 7/10), amoxicillin (n patient = 4/10), PPL (n patient = 5/10), MDM (n patient = 7/10), aspirin (n. patient = 4/11), ibuprofen (n. patient = 4/11), paracetamol (n. patient = 6/11), diclofenac (n. patient = 5/11), lidocaine (n. patient = 3/4) and cefalosporin (n. patient = 4/4). *Conclusion:* these results suggest that flowcytometric BAT could help in the in vitro diagnosis of multiple allergy drug syndrome.

Treatment

Isoniazid-caused patient died in a 48-year-old woman with the Pleuritis tuberculosa

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Poster

The author report on a 48-year-old woman with the Pleuritis tuberculosa where the Isoniazid-induced died. Daily doses of Isoniazid were 1 tablet per day. (Therapy dates: from 25. Decembre 2008 to 28. January 2009; Therapy duration: 35 days). Patients took the following therapy: rifampicine, tab. 300 mg, isoniazid, tab. 300 mg, ethambutol, tab. 400 mg, pirazinamid, tab. 400 mg and propranolol, tab 80 mg. Problems in the form of nausea, vomiting, loss of appetite and pain in the upper part of abdomen were 15. January 2009. The objective of this report is notification a public health about the serious undesired reaction which ended lethal. On receipt of 27. January 2009, the laboratory analysis is set high level transaminase, gamma-glutamyl transpeptidase, and bilirubin (AST-3723 U/L, ALT-4311 U/L, gamma GT-354 U/L, bilirubin 198 umol/L). During hospitalization, there was the development of clinical picture of acute liver failure and patient died 15 days after receipt. It is not the presence of antibodies to hepatoviruse or is there another explanation of damage hepatocytes. Antituberculotic are off the next day upon receipt. Analysis of the case report shows that it is a serious undesired reaction, serious in terms of causing the death of the patient.

Cutaneous NSAID intolerance does not prevent the intake of normal doses of NSAID

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Poster

Background: Immediate non allergic hypersensitivity reactions induced by non-steroidal anti-inflammatory drugs (NSAID), so called "NSAID intolerance", concerns 5% of the general population. Withdrawal of NSAID can be harmful for patients, with no equivalent alternative treatment. In this study, we investigated the tolerability and safety of a normal dose of a NSAID with or without premedication, in patients with a well-documented history of cutaneous NSAID intolerance, expressing as urticaria and/or angioedema. *Material and methods:* A single-placebo-controlled oral challenge procedure with various NSAID drugs was applied to 65 patients with a history of NSAID-induced urticaria/angioedema and negative skin prick and intradermal tests. *Results:* A total of 64/65 (98%) patients tolerated a challenge with a normal dose of NSAID without adverse reactions, using a step-by-step protocol. Ninety percent (59 patients) of oral challenges were well-tolerated with no premedication. Of the 6 patients who experienced reactions to a NSAID alone, a second challenge was performed with premedication by antihistaminics. The response to oral challenge was negative in 2/5 patients (corresponding to 94% well-tolerated challenges at this stage). For the 4 (6%) remaining patients, a challenge with a double premedication by antihistaminics and antileucotriens was carried out and tolerated by 3/4 patients. Only a moderate urticaria occurred in 1 patient, despite the double premedication. Finally, nimesulide, which was used as an alternative drug in 9/65 patients, was tolerated in all cases without premedication. *Conclusion:* Treatment by NSAID at normal doses, associated with premedication by antiH1, is possible and well-tolerated in patients who have experienced NSAID-induced urticaria/angioedema. In cases of cutaneous relapse in spite of the antihistaminic, a double premedication by antihistaminics and antileucotriens seems then mostly effective. Interestingly, nimesulide (as a replacement of the culprit NSAID) is also a safe and well-tolerated alternative.

A case of angiotensin receptor blocker- induced angioedema treated with C1 esterase inhibitor

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Poster

Background: Although angiotensin receptor blockers (ARBs) do not inhibit bradykinin degradation, angioedema has been reported in association with their administration. The mechanism behind this angioedema remains unknown. *Methods:* We report one of the first cases in North America of a patient with ARB induced angioedema responsive to C1 esterase inhibitor. A 65 year old male with a history of hypertension, lung cancer, chronic obstructive pulmonary disease and alcoholism presented with acute onset of ocular swelling, swelling of the lips, and cheeks along with throat tightening for the first time, within 40 days of initiation of Losartan Potassium (Cozaar®). *Results:* Clinical evaluation revealed progressive facial angioedema without dyspnea, increased work of breathing, chest pain or dizziness. C1 esterase inhibitor level and function were within normal limits. The patient was unresponsive to treatment with intramuscular epinephrine, corticosteroids as well as H1 and H2 antihistamines. 2 hours after administration of 500 units i.v of C1 esterase inhibitor, lip swelling and dysphagia resolved. *Conclusions:* ARB induced angioedema is a potentially life threatening complication that requires immediate medical attention. C1 esterase inhibitor should be considered as a possible adjunctive treatment in cases of ARB-induced angioedema when other emergency measures fail.

Systemic or topical corticosteroids in the treatment of dress. Retrospective analysis of 42 cases from a single center

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Poster

Background: DRESS (Drug Reaction with Eosinophilia and Systemic symptoms) is a rare and severe form of drug reaction. It is usually treated with high dose systemic corticosteroids. Because of growing evidence that virus reactivation is frequent and linked to the severity of DRESS we had decided in our department to avoid using corticosteroids or immunosuppressive drugs in absence of life-threatening situation. *Patients and methods:* Retrospective analyze of all patients hospitalized in our department from March 2005 to April 2009 and given a discharge diagnosis of DRESS. *Results:* 42 cases were reviewed (22 females, 20 males, mean age 52 ± 21). Three patients already received systemic corticosteroids before admission for an associated disease. With the intention to treat DRESS, 9 more patients were administered systemic corticosteroids, 1 IVIG (inclusion in a multicenter protocol that was prematurely stopped because of side effects) and 29 received only very potent topical steroids (clobetasol propionate). The reasons for using systemic corticosteroids had been renal failure (5), respiratory distress (2), neuritis (1) fulminant hepatitis (1) non septic shock (1). No patient died. In the group treated by systemic steroids complications and relapses were more frequent and the lengths of hospital stay and of treatment were much longer. *Conclusion:* Since the choice of systemic versus topical corticosteroids had been obviously directed by the severity of the cases on admission, these observations cannot suggest any advantage of topical treatment. Anyhow our experience provides several interesting pieces of information. It shows that a large proportion of DRESS cases can be cured with topical steroids only when they present without life-threatening involvement of kidney, lung, heart or CNS (for which we still recommend systemic corticosteroids). Concerning liver, the most frequently affected organ after the skin there is no evidence that systemic steroids are effective. Our data also strongly suggest that the consensus attitude to treat DRESS with high-dose systemic steroids should be challenged and that randomized trial comparing topical to systemic corticosteroids would not be unethical.

Plasmapheresis for the treatment of severe cutaneous adverse reactions (SCAR) in our department

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Poster

Our standard treatment of severe cutaneous adverse reactions (SCAR) such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) is high-dose of corticosteroid therapy including steroid pulse, which is usually adopted in the first choice. Recently, we have experienced patients with SCAR, who were not controlled even by the high-dose of steroid therapy. Plasmapheresis composing of simple plasma exchange (PE) and double filtration plasmapheresis (DFPP) is well-known as an effective treatment for the SCAR, which can not be controlled by the steroid therapy. But the mechanism of its effect is not yet elucidated enough and this treatment is not widely accepted. In order to evaluate the effects of the treatments, we analyzed 6 cases of SCAR (4 patients with TEN, a patient with SJS, a patient with severe EEM type drug eruption) experienced in our hospitals, from 2000 to 2008. All patients were treated with high-dose corticosteroid such as 250-1000 mg/day of methylprednisolone (mPSL) or 6-10 mg of betamethasone in primary stage. 6 of 5 patients had several complications such as diabetes, hypertension, epilepsy and gastric ulcer. In the process of the treatment, 5 patients had bacterial or viral infections after high-dose of corticosteroid therapies, although early medication of antibiotics or intravenous injection of immunoglobulin (IVIG) was done effectively. Plasmapheresis was performed in addition to the primary therapy in early phase, after that, these patients can be immediately recovered. The plasmapheresis and/ or intravenous injection of immunoglobulin (IVIG) were added appropriately for patients, who were not controlled by the high-dose corticosteroid. We also examined the serum levels of cytokines such as IFN-γ, IL-10, TNF-α, andIL-6 in these patients during the progression, exacerbation and remission phase of diseases. The IFN-γ, IL-10 and TNF-α were increased at the early and exacerbation phase, then decreased after the treatment of pasmapheresis. We thought it was useful to measure these cytokines, in order to estimate the effect on treatment in the SCAR.

G-CSF enhances wound healing in severe TEN and should be considered in management of fulminant TEN irrespective of neutropaenia

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Poster

The course in severe TEN may be so fulminant and detrimental that effective immediate management may mean the difference between life and death. The mortality rate in treated patients presently ranges between 25-40%. If the patient survives, delayed cutaneous and mucosal healing results in abnormal epithelial repair with potentially pronounced mucosal adhesions and cutaneous scarring. To date, trends in promoting wound healing in TEN have been based on provision of supportive care for optimal conditions to allow for physiological repair. These include: clean environment, air mattress, wound dressings, adequate room temperature, fluid and nutritional support and care of any complications. They are of paramount importance and should be strived for. Nonetheless such repair is tardy and in very severe TEN so slow that it may compromise full recovery, accounting for imminent early and late complications with particularly significant mucosal strictures and scarring. To date, management of severe TEN has been directed at arresting the allergic reaction and treating the complications. In recent years there has been interest and debate over the role of steroids, ciclosporin and immunoglobulins as immunosuppressive therapy. Specific medical interventions to accelerate epithelial regeneration have not previously been used. We observed that in two severe cases of TEN, dramatic re-epithelialisation and recovery coincided with the introduction of G-CSF for neutropaenia1. The mechanism by which G-CSF accelerates re-epithelialisation is unknown. Some possible explanations come from basic research. Delayed and altered wound healing has been shown in genetically modified GM-CSF 'knock-out' mice (GM-CSF KO) compared with wild type (WT) mice skin2. The impaired healing in GM-CSF KO was characterised by delayed re-epithelialisation and excessive collagen deposition with delayed healing and prominent scarring. We recommend that G-CSF should be considered for treating severe TEN, irrespective of neutropaenia to promote wound healing and accelerate recovery.

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Clinical features of Toxic Epidermal Necrolysis (TEN) with Chelsea and Westminster TEN management protocol

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Poster

TEN is a rare, but most dramatic, life threatening drug induced allergic reaction known. With a prevalence of 2 per million persons a year it is still likely to be encountered yearly in any big centres. It has 30-40% mortality rate. Familiarity with the clinical features is important in order to make an early diagnosis. Immediate management is a challenge as the sufferer can be anyone from a premature neonate, pregnant woman1 or a middle age or elderly individual with all their background co-morbidities. Almost any drug can cause hypersensitivity reactions and any drug introduced within 1 to 8 weeks from presentation should be considered as a trigger and stopped. Prodromal stage is invariably a feature of TEN. Fever, stinging eyes, pain on swallowing, various discomforts mimicking upper respiratory or urinary tract infections are common presenting features. The skin becomes inflamed and blisters classically appear over the pressure points, in particular upper back and upper arms. Nikolsky sign is likely to be positive (gentle pressure over skin causes epidermal detachment). Mucosal involvement is a norm with erosions of the oral, ocular and genital mucosae in most patients. The epithelium of respiratory, gastrointestinal and urinary tract mucosae also occurs 2. Patient may be extremely unwell with confusion or agitation through pain and high fever. The course is so rapid that appropriate immediate management may mean difference between life and death. ABC Management Strategy 3: A) Arrest of allergy. All potentially implicated drugs should be discontinued immediately. In particular, any drugs introduced within 1-8 weeks of presentation. Immunosuppressive therapy is likely to be beneficial in arresting allergy if commenced early. B) Boost of bioregeneration. Encourage and accelerate re-epithelialisation in order to hasten recovery from TEN and minimize complications. G-CSF achieves this through enhanced re-pithelialisation immunomodulation and immunotolearance 3. C) Care of complications. Patients are admitted to ITU/Burns units for the best supportive care. Temperature, hydration, NG feeds, respiratory supports, are all in place. Complications such, as infections, are treated if and when they occur.

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Clinical cases

A case of drug induced immune thrombocytopenia with ethosuximide

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Poster

Drug-induced thrombocytopenia can be triggered by a wide variety of drugs. Many, if not most, of these medications induce thrombocytopenia by immune mechanism. The present case is a child with ethosuximide induced immune thrombocytopenia. The patient was a six years old boy treated with ethosuximide for absence seizure by neurologist. On the 20th day of administration of etosuximide, he

developed purpuric rash on his extremities, and epistaxis. Simultaneously, he had a fall and trauma to his head and face. Blood test revealed severe thrombocytopenia. CT scan of the brain revealed intraparenchymal hemorrhage in the right frontal lobe. Increased megakaryocytes were seen on the bone marrow aspiration smear. We promptly discontinued ethosuximide, and started intravenous immunoglobulin (1 g/kg/day) for 2 days, and platelet transfusion was performed. On the 2 th days of admission, his platelet count rapidly increased, and after 4 days it became normal. Ethosuximide has various side effects, including hematological effects, but thrombocytopenia is extremely rare. However, close follow up, and CBC testing is advised for early detection of this finding and prevention of further morbidity through stopping the administration of this drug.

Nicolau Syndrome by penicillin injection

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Poster

Introduction: Nicolau syndrome also known as livedo-like dermatitis or embolia cutis medicamentosa is a rare adverse reaction of intramuscular and intra-articular drug injection. The typical presentation is pain around the injection site soon after injection, followed by erythema, livedoid patch, haemorrhagic patch, and finally necrosis of skin, subcutaneous fat, and muscle tissue. *Case report:* A 7 year old boy, a known case of cerebral palsy admitted with fever abdominal tenderness and distension for 9 days. He was received a penicillin injection in the left buttock and one day later developed an echymotic, ischemic and necrotic area in the site of injection which extend to left leg, thigh and foot gradually. Abdominal sonography showed large bowel volvolus so the patient was undergone operation and transverse colostomy was done. In the color Doppler sonography of the lower extremity revealed vasodilation of large arteries, may be due to hyperemia and inflammation. Nicolau syndrome was diagnosed and the patient received prednisolone, pentoxyphyllin and heparinized. In the follow up the lesion began to improve. *Discussion:* The skin reaction is pathognomonic, as exemplified by its synonym "embolia cutis medicamentosa". Localized pathognomonic skin change with history of injection may complete the diagnosis of Nicolau syndrome without skin biopsy. Pathogenesis consist of a secondary vasospasm, intra-arterial injection of solutions intended for intramuscular use, marked inflammation, progressive necrosis of the intima, destruction of the whole arterial wall, necrosis of the skin, precipitatation of crystals and eventually peripheral arterial vessels occlude either through true emboli or through vessel damage. There is no specific therapy for Nicolau Syndrome other than prevention. Tissue necrosis was extensive in our patient so prednisolon, pentoxyphyllin and heparin were used with favorable response.

Temozolomide-induced drug rash with eosinophilia and systemic symptoms

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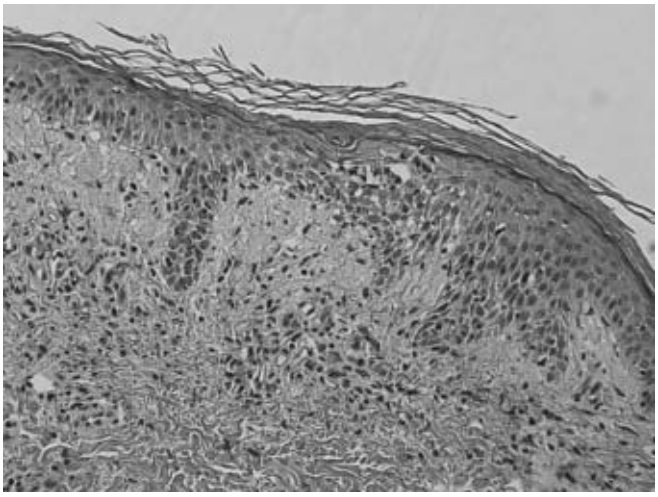
Poster

Introduction: Drug rash with eosinophilia and systemic symptoms (DRESS) syndrome is a rare and potentially life-threatening hypersensitivity reaction comprised of rash, fever, internal organ involvement, and hypereosinophilia. While many medications may result in the development of DRESS syndrome, aromatic anticonvulsants and sulfonamides are most commonly implicated. *Case Report:* We report the case of a 28-year-old man with glioblastoma multiforme who presented with severe rash, fever, eosinophilia after a second cycle of the chemotherapeutic alkylating agent, temozolomide. He was first hospitalized with DRESS syndrome after an initial cycle of Dapsone and temozolomide. At that time, he was ill-appearing and febrile with generalized pruritic erythrodermal lesions, axillary lymphadenopathy, eosinophilia (1800 total eosinophil count, 36%), and elevated liver enzymes (AST/ALT, 192/432). He was successfully treated with a 3 day course of intravenous immunoglobulin (IVIG) 1 mg/kg (Gamunex®). Histopathology of the skin lesions (Figure 1) revealed interface dermatitis with lymphomatoid features, findings typical of DRESS syndrome. During this first admission, his condition was thought to be due to Dapsone, because of the medication's known implication in DRESS syndrome. Upon subsequent admission with similar symptoms, he had restarted temozolomide the day prior; Dapsone had been discontinued since the first hospitalization. His condition improved with prompt discontinuation of temozolomide and intravenous methylprednisolone. *Conclusion:* The diagnosis of DRESS syndrome can be challenging due to both the constellation of clinical and histological features, and the variety of inciting agents. However, once the diagnosis is made, identification and prompt withdrawal of the offending medication is necessary because of potential mortality. Although controversies exist regarding the use of corticosteroids and IVIG, they are still widely used. While certain classes of medication are most commonly associated with the development of DRESS syndrome, the case report literature suggests a broader category of causative agents. To our knowledge, this is the first reported case of temozolomide-induced DRESS syndrome.

Figure 1



T cell immunoreactivity to cefozopran, but not to phenytoin, in the early phase of drug induced hypersensitivity syndrome

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Poster

Drug induced hypersensitivity syndrome (DIHS) is a rare form of drug hypersensitivity that is closely associated with human herpes virus 6 (HHV-6) re-activation. DIHS typically presents as a biphasic disease, first of which phase attributes to the causative drug, and second phase possibly to re-activation of HHV-6. Although the mechanisms of HHV-6 re-activation is not clear, increased numbers of regulatory T cells and decreased total immunoglobulin often observed upon DIHS onset, might reflect immune suppression that could allow virus reactivation. Herein, we report a case of DIHS in a 30-year-old Japanese female with underlying cervix cancer complicated with brain metastasis and recurrent cellulitis. She had been taking phenytoin sodium for 5 weeks before she was started with cefozopran hydrochloride to treat her cellulitis. A week later, she developed fever, cervical lymphadenopathy, liver dysfunction, and maculopapular erythema, which rapidly coalesced and presented as erythroderma. Blood test upon onset revealed eosinophilia and atypical lymphocytes, as well as positive HHV-6 DNA by PCR and elevation of anti-HHV-6 antibodies later in the course, collectively leading to the diagnosis of DIHS. Both drugs were withdrawn and 1mg/kg/day of prednisolone rapidly resolved all symptoms. Interestingly, drug-induced lymphocyte stimulation test (DLST) was initially positive for cefozopran hydrochloride and negative for phenytoin sodium, but both drugs yielded positive results 3 months after disease onset. The DLST results for phenytoin sodium is typical of a causative drug in DIHS, where initial negativity could be explained by the transient immunosuppression that takes place early in the disease phase. However, DLST positivity for cefozopran hydrochloride in the first test indicated the presence of strong T cell immunity against this drug, suggesting that there might be a degree of antigen specificity in immunosuppression observed in the initial phase of DIHS.

Is acute generalized exanthematous pustulosis an uncommon condition in childhood?

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Poster

Background: Acute Generalized Exanthematous Pustulosis (AGEP) is an uncommon condition in children. It is characterized by acute onset of widespread pustular eruptions in association with systemic symptoms and usually seen as a drug reaction. *Methods:* We present four children ages range 6-16 with AGEP possibly secondary to exposure medication in the last one year (Table 1, pg. 45). *Conclusion:* It should be thought AGEP when erythematous eruption covered with numerous follicular pustules are seen in childhood. To our knowledge, this is the first report from Turkey in children.

Drug-induced hypersensitivity syndrome and subsequent arthritis

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Poster

Background: Drug-induced hypersensitivity syndrome (DIHS), also known as drug rash with eosinophilia and systemic symptoms (DRESS), is a rare but severe reaction to drugs, and may be related to the reactivation of herpes viruses. It is characterized by skin rash, fever, lymphadenopathy, leukocytosis with eosinophilia, internal organ involvement, and HHV-6 reactivation. We recently observed the unique patient with mexiletine-induced hypersensitivity syndrome, who developed symmetrical arthritis after the resolution of skin rash. *Materials and Methods:* A 64-year-old Japanese woman with arrhythmia developed a fever and maculopapular rash over her face and trunk 4 weeks after the initiation of oral mexiletine. At first withdrawal of mexiletine and intravenous hydrocortisone did not improve her eruption, and she was hospitalized on 5 January 2009. Laboratory studies showed leukocytosis (15,900/μl) with atypical lymphocytes, and liver dysfunction (AST 100 U/L, ALT 182 U/L). On the 14th day of hospitalization, polymerase chain reaction detected the HHV-6 DNA (3.4x102 copies in 1 μl of whole blood), but no other herpes viruses. A skin biopsy demonstrated lymphocytic infiltration in the epidermal-dermal junction and liquefaction degeneration of the basal cell layer. Patch test and drug-induced lymphocyte stimulation test demonstrated positive reaction to mexiletine. We diagnosed her with mexiletine-induced hypersensitivity syndrome. Oral prednisolone at 40 mg /day was effective in improving skin eruption, fever, and liver dysfunction, and prednisolone was weaned carefully. The bilateral wrists and knees were slightly swollen with pain after prednisolone dose was reduced from 20 to 15 mg/day on 5 March. Serum MMP-3 was increased to 582 ng/ml on 27 May, which was in the normal range 17.8 ng/ml on 13 January. *Conclusion:* This is the first case of DIHS associated with arthritis, which may result from immune system dysregulation by drug allergy and/or HHV-6 reactivation.

Non immediate allergic reaction to Infliximab. Immunological study of a case report

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Poster

Tumor necrosis factor-α (TNF-α) is an important inflammatory cytokine in many rheumatic diseases. New treatments for these diseases include the use of TNF-α antagonists as adalimumab which is human form of monoclonal antibody and infliximab which is a chimeric IgG1 monoclonal antibody. These new biologic response modifiers can produce adverse reactions, both predictable and unpredictable both moderate as urticaria and other more severe as Stevens Johnson Syndrome or toxic epidermal necrolysis. We present a case report of a 39 years old woman with rheumatoid arthritis and Chron disease that developed a generalized maculopapular exanthema seven days

Table 1 - Clinical characteristics of our four patients

	Case 1	Case 2	Case 3	Case 4
Age (year)	9	6	11	16
Gender	Male	Male	Female	Male
Primary diagnosis	Rectal atresia with colostomy	Acute laryngitis	Acute appendicitis	Volvulus with colostomy
Drug usage	SAM, Clindamycin, Amicasin	Cefixime,metil PRD	SAM, Clindamycin, Amicasin	Ampicillin, Clindamycin Amicasin
History of pustular eruption	First day	Sixth day	Third day	Third day
Clinical characteristics	Maculo-papular eruptions on the face. Erythematous eruption covered with numerous follicular pustules on the trunk, and abdomen	Erythematous eruption covered with numerous follicular pustules on the face, and extremites	Erythematous eruption Erythematous eruption covered with numerous follicular pustules and maculo-papular eruptions on the trunk and proximal of extremites	Erythematous eruption covered with numerous follicular pustules on the trunk and extremites
Fever	+	+	+	+
Leucocyte	11.900/µL	14.300/µL	23.000/µL	10.200/µL
Eosinophil (%)	4	8	8	6,5
Liver-kidney function tests	Normal	Normal	Normal	Normal
Viral serology	Negative	ND	Negative	Negative
Treatment	Antihistamines	Antihistamines	Antihistamines	Antihistamines
Histopathology	Acanthosis in epidermis Lymphocytic spongiosis Spongiform pustules, perivascular neutrophils infiltrate	Epidermal pustules, Necrotic keratinocytes, perivascular neutrophils infiltrate	ND	ND
Patch test Clindamycin:	Ampicillin: POSITIVE	Steroid: Negative Cefixime: Negative	ND	ND

SAM: Sulbactam-ampicillin PRD: Prednsinolone ND: Not done

after beginning treatment with Infliximab (150 mg). The drug was stopped, steroids treatment initiated and the exanthema disappeared in five days. *Methods:* Two months after an allergollogical study was done including intradermal test with Infliximab (1/10000) and if negative single blind placebo controlled drug provocation test (DPT). The immunological study after DPT included the monitorization of the response (T1, T2 and basal) by flow cytometry analysis of different cellular markers and a skin biopsy obtained at the acute phase of the reaction. *Results:* The intradermal test was negative at 48 and 72 h. reading. DPT was positive, with the patient developing a maculopapular exanthema 24 hours after the administration of 15 mg of infliximab. The immunological study showed an increase of CD3+CD4+ lymphocytes expressing CXCR3, CLA and CCR10 indicating a Th1 pattern. With respect to the cytotoxic markers we observed a strong increase in the perforin content in both T and NK lymphocytes. These results were confirmed in the skin biopsy. *Conclusions:* This case report showed a non-immediate reaction to infliximab. The monitorization of the immunological response showed the involvement of T lymphocytes with skin homing and a Th1 pattern with cytotoxic characteristics.

Severe bullous hypersensitivity reactions after exposure to carbamazepine in Han Chinese child with positive HLA-B*1502 and negative lymphocyte toxicity assay: pathophysiological considerations

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Poster

Drug hypersensitivity syndrome (DHS or DRESS) is a rare but potentially fatal disorder occurs in susceptible patient following exposure to certain drugs including aromatic anticonvulsants (AACs),

non-steroidal anti-inflammatory drugs (NSAIDs), antimicrobials and others. The exact pathophysiology underling DHS is not well understood and it is thought to involve two major components: drug biotransformation and immune system activation. Carbamazepine-induced DHS (CBZ-DHS) can be presented as several clinical forms ranging from simple maculopapular skin rash to severe bullous reaction and multi-system dysfunctions. Genetic analysis of CBZ-DHS patients has revealed striking association relationship between CBZ-induced severe bullous reactions such as Steven-Johnson syndrome and Toxic Epidermal necrolysis (CBZ-SJS/TEN) in Southeast Asian populations and carrying a specific HLA allele (HLA-B*1502). This ethnic specific relationship with certain disease phenotype has raised the question on the commonality of the pathogenesis mechanisms of the diseases. Here we present a typical case of CBZ-SJS in a child of Han Chinese origin who was found to be HLA-B*1502 positive. We performed two types of in vitro toxicity assay, the lymphocyte toxicity assay (LTA) and the in vitro platelet toxicity assay (iPTA) on cells taken from the patient and two healthy volunteers 3 and 9 months after recovery from the reaction. We also tested a typical CBZ-DRESS patient 3 months after the reaction. Both tests (LTA and iPTA) turned out negative 3 and 9 months from the reaction on samples from the CBZ-SJS/TEN patient. However, the LTA test was positive in the CBZ-DRESS case. These results provide evidence on more than one mechanistic etiology for CBZ-DHS.

Intractable Stevens-Johnson syndrome caused by *Mycoplasma pneumoniae* infection

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Poster

Backgrounds: Stevens-Johnson syndrome (SJS) association with *Mycoplasma pneumoniae* (*M. pneumoniae*) infection is mainly observed in children. Adult patients with SJS are mainly due to drugs but some of them are infected with *M. pneumoniae*. We analyzed SJS associated with M. pneumoniae infection in order to elucidate the clinical characteristics of SJS associated with *M. pneumoniae* infection. *Methods:* We presented an adult case of intractable SJS associated with *M. pneumoniae* and cytomegalovirus reactivation. In addition, data of patients who have been reported as SJS associated with *M. pneumoniae* in medical Journals from 1981 to 2008 were analyzed retrospectively, in comparison with data of patients reported as SJS due to drugs from 2000 to 2009 in Japan. *Results:* Case report: A 33 year-old man was admitted to our hospital because of a fever, painful erosions on the eyelids, conjunctiva, lips, oral mucosa and penis, and many macules on the palms and soles. Eight days before that, he had had a fever and dry cough. Skin biopsy taken from macular lesion showed many of apoptotic epidermal cells with a mononuclear cell infiltrate. Laboratory examination showed an increase of anti-*Mycoplasma* IgG antibodies with 1280 times by particle agglutination method. He had treated with antibiotics and systemic corticosteroids including pulse therapy. However, his symptoms did not subside and erythematous lesions appeared on his trunk accompanied by liver dysfunction. After intravenous injection of immunoglobulin (5 g/day for 3 days), the mucosal lesions were ameliorated gradually but the skin lesions were still observed even 5 months after the beginning of the symptoms. During the course, his symptoms were exacerbated with a detection of cytomegalovirus antigen in the serum. The serological test showed an increase of cytomegalovirus IgG titers by ELISA from 9.7 to 82.9 after the exacerbation. Thirty-eight *M. pneumoniae*-associated SJS and 78 drug-induced SJS were analyzed in this study. The mean age was 15.3 years old of the patients with *M. pneumoniae*-associated SJS and 43.1 years old of the patients with SJS, In comparison with drug-induced SJS, more patients was improved without corticosteroid therapy and organ involvements were less in *M. pneumoniae*-associated SJS. *Conclusion:* SJS associated with *M. pneumoniae* infection may be milder than SJS induced by drugs in children, but it might be intractable in adult patient by some factors including cytomegalovirus reactivation.

Amikacin-induced DRESS: usefulness of skin tests and immunobiological ELISPOT assays

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Poster

Delayed Allergic hypersensitivity to aminoglycosides antibiotics is rare. Only a few cases of severe skin reactions as Lyell's syndrome have been reported. We report a case of drug hypersensitivity syndrome (DIHS) following treatment with amikacin which, to our knowledge, has not been yet reported. In 2004, a 42-year-old man, received clindamycine, amikacine and vancomycine for septic arthritis of the right knee. Eighteen days after starting treatment he presented maculopapular and oedematous skin reaction, facial edema and fever. Blood tests showed hypereosinophilia upper 8000/mm3, increased liver enzymes (ALAT upper 11 N) and coagulation disorders (prothrombin time=30%).Viral serologies and blood polymerase chain reaction were negative. In the suspicion of DIHS these drugs were stopped, topical corticoids and systemic treatment by prednisolone 60 mg/day were started. Under this treatment desquamation followed and the patient improved slowly until complete remission 1 month later. In January 2009, skin patch tests were performed, with the different drugs (clindamycine, amikacine and vancomycine) prepared by the hospital pharmacy and provided as 10% serum saline solution. Amikacin gave a positive reaction at 48 and 72 h readings. All other drugs gave negative results. Detection of circulating specific T cells, analyzed by interferon-γ (IFN-γ), IL17 and Granzyme B enzyme-linked immunospot assay (ELISPOT) was positive for amikacin and negative for clindamycin and vancomycin. The diagnosis of T cellmediated allergic hypersensitivity to amikacin was made. Fourty eight hours after removal of the patch tests, the patient experienced a disease flare without any blood disorders (renal, hepatic and blood count cell). Topical steroids, moisutrizers and antihistaminics led to complete remission in 7 days. In order to evaluate cross-reactivity with other aminoglycosides, the patient received, in September 2009, a second series of epidermal tests to gentamycin, netilmycin, tobramycin, neomicyn and spectinomycin. Patch tests were positive with amikacin only.

Gentamycine reintroduction was performed in September 2009 without any hypersensitivity reaction. Our case suggests that skin test and ELISPOT assay could be of value for the diagnosis of aminoside-induced DIHS and for defining a non cross-reactive aminoside that could be given safely to the patient. Elispot could also avoid potentially lethal skin tests in these cases.

Amoxicillin-induced exanthema in patients with infectious mononucleosis

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Poster

Background: It is known that amoxicillin enhances the incidence of exanthema in patients with EBV-induced mononucleosis. However, the exact mechanism for these eruptions is unclear. The maculopapular exanthema could be explained by a virus-dependent rush, transient decrease in drug tolerance during virus infection or a true allergic drug reaction. *Materials and Methods:* We report four young adults with amoxillin-induced flare during an episode of infectious mononuleosis. Our patients were presented with maculopapular exanthema, lymphadenopathy and all of them had a history of fever and sore throat treated with amoxicillin-clavulanic acid for one week before admission. Laboratory investigations revealed lymphocytosis, elevated liver enzyme levels, and significantly increased Epstein Barr virus specific immunoglobulin M antibodies. *Results:* The patients were diagnosed with infectious mononucleosis. One patient was further investigated to see whether the amoxicillin-induced exanthema was a virus-associated phenomenon or drug hypersensitivity. After a negative lymphocyte transformation test (LTT), prick test showed negative result with the major determinant benzylpenicilloil poly-L-lisine, but the intradermal test with the major determinant was positive in a dilution of 1:10 after 20 minutes indicating drug allergy. *Conclusion:* Our data provide additional evidence that true allergic reactions to aminopenicillin may develop during a viral infection and drug allergy tests are helpful to identify sensitized patients.

Diluition of iodinated contrast material for intradermal testing of reactors

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Poster

Background: Skin testing of patients who have immediate hypersensitivity reactions to iodinated contrast material (ICM) is questioned. False positive results are anticipated with concentrated solutions, due to hyperosmolality or to chemical toxicity. ICMs are used diluted up to 1:10, which do not elicitate positive intradermal tests (IDT) in normal subjects (1). IDT with undiluted ICM solutions may be necessary, as shown by the following case. *Materials and methods:* IDT were performed with diluted or pure ICM and were considered positive where a pruritic red papula, doubling the injection papula, was observed. *Results:* A female patient, aged 55, was injected Optiject (ioversol) 100 ml after a 3-day pretreatment with anti-histamine and glucocorticoid. Generalized urticaria, angioedema and dyspnoea appeared ten minutes after. She recovered within 2 hours. She had a history of a similar reaction 20 years before, but had received ICM uneventfully afterwards. Six months after, IDT were positive with ioversol 1:10000. Skin cross-reactivity was observed with iomeprol 1:1000, iohexol 1:100, ioxaglate and iopromide 1:10. Five other ICM, including iodixanol gave negative IDT up to dilution 1:10. Seven months after, she was injected Visipaque (iodixanol) after a 3-day pretreatment with hydroxyzine. Five minutes after, she developed generalized erythema, dyspnoea, and tremulations. Plasma histamine and tryptase concentrations were moderately increased. Skin tests were performed 8 days after. The same IDT results as the first ones were obtained for iomeprol 1:1000, and for the 5 negative ICM 1:10. When pure solutions were tested, iodixanol and 2 other ICM (ioxitalamate, amidotrizoate) gave positive IDTs. Two ICM (iopamidol, iobitridol) remained negative. The patient was injected Xenetix (iobitridol) one year later, after pre-treatment with hydroxizine, no reaction was seen. *Conclusion:* Intradermal testing with ICM diluted 1:10 failed to identify cross-reacting ICMs, as shown by the recurrence of a similar reaction in this patient. IDTs with pure ICM solutions should be investigated for the diagnosis of immediate hypersensitivity reactions. The study of control subjects should be encouraged to determine the specificity of IDT with pure ICM solutions.

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Hypersensitivity reactions to drugs: a retrospective analysis of

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Poster

Introduction: Drug hypersensitivity (DH) constitutes one of the most frequent reasons for consultations in allergology services with an increasing prevalence in recent years. There are few true epidemiological data on DH. Objectives: To describe the clinical characteristics of patients with suspected DH. *Methods:* The medical records of 20 consecutive patients with suspected DH, enrolled from a large retrospective study designed to include about 400 patients consulted in a Norwegian Allergy Centre, were investigated with respect to history, skin tests and serology. *Results:* Mean age was 43.25 years (range12-84), and 70% were females. 13 patients (65%) had a previous allergic history, including pollen allergy and/or allergy to other allergens, 8 of them (62%) had also a previous reaction to drugs. The drugs most often suspected were NSAIDs (65%), antibiotics (40%), and paracetamol (25%). 53.8% of those who had reactions to NSAIDs had also reactions to antibiotics and/or paracetamol. Cutaneous symptoms (37.5% urticaria) were most frequently reported (60%). Quincke's edema, respiratory, gastrointestinal and circulatory symptoms accounted for 50%, 35%, 35% and 30% of the symptoms, respectively. Anaphylaxis was reported in 10 patients, and 45% of the reactions occurred within 1 hour after taking the drug. Total serum IgE was increased (> 120 KU/L) in 40% and serum ECP was increased (> 22.0 μg/L) in 20% of patients. 3 patients had antigen-specific IgE antibody concentration above 0.35 KU/L (2 to penicillin and 1 to

morphine/pholcodine). Skin prick tests (SPT) were performed with suspected drugs in 19 patients. Only 1 patient (5.3%) had a positive SPT to morphine/codeine, 17 (89.5%) had negative results and 1 patient had inconclusive SPT. Open oral provocation tests (PT) with suspected drugs were performed in 7 patients of whom only 1 patient had a delayed reaction to penicillin, the remainder was negative. *Conclusions:* Suspected DH reactions occur most frequently in female patients, and in those who have a previous history of allergic reactions. The most common manifestations are cutaneous symptoms, but life-threatening reactions may occur. NSAIDs and antibiotics are the two drug families most frequently suspected. These preliminary results are based on a limited number of patients and may not be extrapolated to the general population. PT needs to be included in diagnostic protocols in order to evaluate suspected DH reactions.

Immediate hypersensitivity to moxifloxacin with tolerance to ciprofloxacin

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Poster

Background: Moxifloxacin is a third generation fluoroquinolone with markedly improved Gram-positive activity that is commonly prescribed for respiratory tract infections. Unpredictable immediate hypersensitivity reactions have been reported with moxifloxacin despite its overall safety profile. We report three cases of immediate hypersensitivity reactions to moxifloxacin in patients who tolerated ciprofloxacin. *Case Summaries:* Three patients with a prior history of a moxifloxacin reaction developed an immediate hypersensitivity reaction upon oral challenge with moxifloxacin in our Drug Safety Clinic. The reaction was mainly characterized by pruritus and urticaria, although dyspnea and hypotension were noted in two patients. Two of the patients had negative oral challenge tests to ciprofloxacin and all three patients tolerated full treatment courses of oral ciprofloxacin. *Discussion:* Previous publications have reported both cross-reactivity and lack of cross-reactivity amongst various fluoroquinolones. The three patients discussed herein demonstrated a lack of cross-reactivity between moxifloxacin and ciprofloxacin since they tolerated oral challenge tests and full treatment courses of ciprofloxacin. Moxifloxacin has unique side chains at positions 7 and 8 on its bicyclic ring structure. Antigenic specificity to particular side chains at positions 7 and 8 on the bicyclic ring structure of moxifloxacin may possibly explain this lack of cross-reactivity. Higher reporting rates of anaphylaxis to moxifloxacin compared to other fluoroquinolones may also be related to side chain specificity, although definitive evidence for this is lacking. *Conclusions:* Based on our experience, patients who develop immediate hypersensitivity reactions to moxifloxacin may receive ciprofloxacin therapy in an appropriately monitored setting, if they have previously tolerated full treatment courses of ciprofloxacin. Further research into whether there is a specific side chain reaction unique to moxifloxacin alone is warranted.

A case of acute generalized exanthematous pustulosis (AGEP) induced by hydroxyzine pamoate (HP) and followed by psoriasis vulgaris

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Poster

An 81-year-old man was referred for generalized erythema with high fever to our hospital. He had taken an antihistamine, HP for 7 days and next day he developed itchy eruption on his back, chest, and limbs. The eruption had spread gradually on his whole body during next 8 days. On admission, he had desquamative erythema and red papules on his whole body associated with many aseptic pustules. The eruption was associated with fever of 38°C, and lumbar and finger pain. Laboratory examination showed leukocytosis of 18400/mm^3 and neutrophilia of 81.4%, and elevated level of C-reactive protein of 10.97 mg/dL. Histology of a skin biopsy specimen from his abdomen showed a subcorneal microabscess with neutrophils. No findings of vasculitis were found. He was treated with amoxicillin 750 mg/day, and his eruption and other symptoms were disappeared in 10 days. Patch testing with HP showed a positive result with erythema and pustular formation. Thus he was diagnosed as acute generalized exanthematous pustulosis (AGEP) caused by HP. However, thirty days after his recovery, he developed psoriasis vulgaris, which continued for 1 year until now. Cytokines in serum were measured at some points during the clinical course. Many cytokine levels including Il-6, IL-8, IL-12, IL-17, TNF-α, interferon-γ and GM-CSF rose at the time of admission for AGEP. It is thought that AGEP developed in the patient might be relevant to the induction of psoriasis though an elevation of cytokines including IL-8 and IL-17, which are key cytokines for psoriasis vulgaris.

Stevens-Johnson syndrome and lamotrigine: a case of negative rechallenge

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Poster

Background: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe skin reac-tions with a high morbidity and mortality. They present with widespread exanthema followed by blisters and skin detachment in addition to hemorrhagic erosions of mucous membranes. A few medications are associated with a high risk for SJS/TEN, e.g. allopurinol, anti-infective sulfonamides, carbamazepine, lamotrigine, nevapirine, oxicam-NSAIDS, phenobarbital and phenytoin. A delay of 4-28 days between beginning of drug use and onset of the adverse reac-tion is the most suggestive timing supporting drug causality in SJS/TEN. *Materials/Methods:* We report on a case of SJS in a 53 year old woman who suffered from psychiatric disorder, epilepsy and chronic obstructive pulmonary disease. The patient developed swelling of her lips and eye-lids, one day later skin lesions. Within four weeks before these symptoms, the patient has received 17 different drugs. *Results:* Based on the algorithm for causality assessment in SJS/TEN (ALDEN), lamotrigine could be identified as the "very probable" inducing agent. Acetylcysteine, am-

broxol, quetiapine and a potassium containing combination drug were considered as possible causes. Lamotrigine was initiated 15 days before the onset of the reaction starting with 12.5 mg and a dosage increase up to 50 mg per day. It was stopped two days after the onset of the adverse reaction which was diagnosed as SJS with generalized exanthema and hemorrhagic erosions of mouth, lips, nose, anus, vulva and conjunctivitis. She received intravenous prednisolone for 5 days (250 mg tapered to 50 mg/d) followed by 60 mg prednisolone orally tapered over 16 days. Six days after discontinuation 50 mg lamo-trigine were introduced again while the patient was still on prednisolone therapy. Lamotrigine was tolerated and 24 days after the onset of SJS the patient was discharged. *Conclusions:* Tolerance of lamotrigine after re-exposure was an unexpected finding, since lamotrigine is known to have a high risk for SJS/TEN, whereas for the concomitant medication no risk could be identified through epidemiologic studies. After the retrospective application of ALDEN taking the negative re-challenge of lamotrigine into account, the risk of lamotrigine was downgraded. However, it remains unclear, whether one of the low risk drugs caused the reac-tion or whether lamotrigine was the culprit drug that was later tolerated due to some sort of desensitization under prednisolone therapy.

Acetylsalicylic acid provocation test and desensitisation in a cardiac patient with a history suggesting acetylsalicylic acid hypersensitivity

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Poster

Background: Acetylsalicylic acid is a widely used drug for patients with chronic cardiovascular disease, including coronary artery disease. Long term aspirin therapy prevents platelet aggregation and reduces mortality in cardiovascular patients by irreversibly inhibiting platelet cyclooxygenase-1. Many patients are denied treatment with acetylsalicylic acid because of a history of acetylsalicylic acid-induced urticaria or angioedema. The aim of the study was to determine the outcome of one day protocol for acetylsalicylic acid provocation test and desensitisation. *Materials and method:* We report a case of acetylsalicylic acid hypersensitivity in a 45 years old male patient, known with mitral-aortic rheumatic disease and transient ischemic attack, with remitted right hemiparesis, and history suggesting acetylsalicylic acid hypersensitivity characterized by angioedema (swelling of the lips and lower part of the face). The patient was referred to our Allergy Department at the "Victor Babes" Hospital Timisoara for acetylsalicylic acid provocation test and desensitisation. The prick test for aero- and food allergens as well as specific IgE were negative, and total serum IgE ranged in normal limits. A one day protocol for the evaluation of acetylsalicylic acid hypersensitivity was performed using Aspenter tablets of 75 mg. After signing the informed consent, a peripheral venous access line was placed. Emergency resuscitative equipment was available. The drug was administered per os in 5 increasing doses every 90 minutes, beginning with 20.25 mg, and ending with 325 mg (20.25, 40.5, 81, 162.5, and 325 mg). 15 minutes before and after each dose we measured the blood pressure, heart rate and peak expiratory flow. The occurrence of any rhino-conjunctival, respiratory, cutaneous, digestive or cardiovascular reactions was also observed. *Results:* Acetylsalicylic acid desensitisation was successful, reaching a dose of 325 mg (and a combined dose of 629.25 mg) in 8 hours without adverse reactions. The patient has been under treatment with Aspenter for 4 months since the desensitisation was performed, with no events. *Conclusion:* Rapid desensitisation is safe and efficient in patients who require long term therapy with acetylsalicylic acid for cardiovascular disease.

Development of contact hypersensitivity after application of bioocclusive bandage with ibuprofen

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Poster

Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) have been applied topically for decades. This route possibly reduces gastrointestinal adverse reactions by maximizing local delivery and minimizing systemic toxicity. Compared with oral NSAIDs, fewer patients taking topical NSAIDs had any adverse events, withdrawals due to side effects, and gastrointestinal side effects, but significantly more patients had local side effects such as rash, itch, and burning. Aim of this case was to facilitate healing of allergic leukocytoclastic vasculitis lesions – symmetric purpuric exanthema and ulcer caused by immune complex reactions on coutaneous capillaries and venules endotel. *Materials and methods:* A 69 year old woman was admitted with shallow secreting and painful ulcerations on her right leg, and hemorrhagic papulesand vesicules symmetrically distributed on both legs. The histopathologic and immunopathologic examinations confirmed the diagnosis of vesculitis allergic leukocytoclastica. Patient was administered systemic corticosteroids in a dose of 0.5 mg/kg body weight. Topical therapy revealed application of corticosteroid agents, antiseptic dressings on ulcer, enzymatic debridement, which prevents pain and because of gastrointestinal adverse reactions we decided to use local bioocclusive bandage poliuretan-ibuprofen (INN). *Results:* After two weeks of therapy introduction, ulcer defect was filled with granulation tissue, with reduction of size, swelling and redness of the surrounding skin, as well as the level of pain. Contact sensitivity appeared as a result of local application of ibuprofen which we have proved with epicutaneous test so we end with local application of bioocclusive bandage with ibuprofen. The use of antiseptic measures, ulcer covering with bio-occlusive INN dressings in combination with systemic corticosteroid management resulted in ulcer healing in our patients. *Conclusion:* Efficient treatment of allergic leukocytoclastic vasculitis with bioocclusive compression bandage was proven. Instructions about avoiding arylpropinic acid derivatives such as ibuprofen, fenoprofen, and ketoprofen were given to our patient.

Overlap Stevens-Johnson syndrome/toxic epidermal necrolysis: a case report including study of Alfa-defensins, IFN-gamma and FAS-L expression

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Poster

Background: Stevens-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) are the most severe forms of hypersensitivity reactions affecting the skin. Their pathogenesis and treatment are not completely elucidated. *Materials and methods:* A female 39 yrs old patient was admitted in the burn unit in our hospital suffering from severe constitutional symptoms, fever and blisters on the 25% of her skin surface and involvement of her oral, ocular and genital mucous membranes. These features had appeared on the 6th day of treatment with Amoxycillin-clavulanic acid 1 g/8h, paracetamol 1 g/8h and Ambroxol as a pneumonia treatment. Infectious mononucleosis (EBV) had been diagnosed 4 months before. She was diagnosed of overlap SJS/TEN by the dermatologist. SCORTEN: 1. All these drugs were stopped and treatment with Intravenous human immunoglobulins (IVIG) 0.75 g/d was infused for four consecutive days. Serological analytical tests and biopsy were performed. The expression of CD94/NKG2C and of alfa-defensins 1-3 was analyzed by flow cytometry in PBMCs and blister cells from this patient. Soluble alfa-defensins were mesasured by ELISA in plasma and blister fluid. IFN-gamma and Fas-L levels were measured by flow cytometry (CBA). PBMCs were analyzed for their ability to kill HLA-E-expressing cells in 51Cr release assays. Allergological studies including prick, intradermal, epicutaneous and Lymphocyte Transformation Test (LTT) were performed 3 months later. *Results:* On the third day after starting IVIG treatment, cutaneous lesions started to improve. Skin lesions were completely reepithelized after two weeks. As sequelae she presented skin hypopigmentation and ocular dryness. CD94/NKG2C+ T and NK cells were found in peripheral blood and blister fluid cells from this patient. Moreover, PBMCs were able to kill HLA-E-expressing targets in an NKG2C-dependent manner. Intracellular alfa-defensins 1-3 were found in blister fluid cells. In addition high concentrations of soluble alfa-defensins 1-3, IFN-gamma and Fas-L were detected in blister fluid in acute phase. Skin tests were negative for all the drugs tested. LTT was strongly positive for amoxycillin and amoxycillin-clavulanic acid and weakly positive for paracetamol and ambroxol. *Conclusion:* We report a case of overlap SJS/ TEN probably induced by amoxycillin-clavulanic acid. IVIG treatment has been apparently of benefit in our patient. Our analytical findings support the role of CD94/NKG2C + T and NK cells, and of alfa-defensins, IFN-gamma and Fas-L in the pathogenesis of TEN.

Telemedicine for consultations in drug allergy

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Poster

Background: Telemedicine is the delivery of health-related services and information using telecommunications technologies such as two-way videoconferencing systems and when applicable, diagnostic instruments such as digital stethoscopes and patient examination cameras. Telemedicine has been used in various health specialities such as dermatology, ophthalmology and cardiology to monitor and care for patients, especially those who live in remote locations. The Ontario Telemedicine Network (OTN) is one of the largest telemedicine networks in the world, providing healthcare services by enhancing access to health care providers and eliminating barriers to care, especially those patients living in rural and remote areas. *Methods:* The Drug Safety Clinic is a referral center located at Sunnybrook Health Sciences Centre, a large academic health care centre located in Toronto, Ontario that evaluates patients with histories of adverse drug reactions. Sunnybrook has a well established Telemedicine Program within the OTN. Through the Sunnybrook Telemedicine Resources and the infrastructure of the OTN, the Drug Safety Clinic is able to facilitate initial assessments of patients who live at a distance to Sunnybrook to better plan for appropriate diagnostic tests. A review of patients seen at the Drug Safety Clinic through Sunnybrook's Telemedicine Program and the OTN was done. *Results:* 164 patients were evaluated through the Telemedicine Program between February 2006 and November 2009. This compares to 5619 patients seen in-person at the Drug Safety Clinic over the same time period. The average age of the patients seen through telemedicine was 47.8 years (range 7-88 years), with females representing 79% of patients. Patients had histories of adverse drug reactions to an average of 2.7 drugs (range 1 to 20). In 114 (70%) patients, diagnostic testing was considered appropriate and of these, 82 (50%) patients travelled to Toronto for testing. The most common tests scheduled were ASA oral challenge (15 patients), local anaesthetic skin tests (18 patients), and penicillin skin tests (59 patients). Travel that was avoided with the use of telemedicine was approximately 40 000 km. *Conclusion:* In our sample of 164 patients initially seen for consultation, only 50% travelled to Toronto for diagnostic testing. Telemedicine is a viable, eco-friendly option for long-distance consultations, including patients with histories of adverse drug reactions.

Overview of anaphylaxis following ecallantide treatment for acute attacks of hereditary angioedema

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Poster

Background: Hereditary angioedema (HAE) is a potentially fatal disease characterized by unpredictable, acute attacks of nonpruritic edema. Ecallantide is a novel plasma kallikrein inhibitor developed for the treatment of acute HAE attacks. Anaphylaxis (both anaphylactic and anaphylactoid reactions) was reported during the ecallantide development program. An analysis of these reactions is presented. *Method:* Ecallantide was evaluated for HAE in 7 clinical studies, including 2 double-blind, placebo-controlled, Phase 3 studies. Both intravenous (IV) and subcutaneous (SC) dosing were used. The appropriate IRB approved each study; all patients provided written informed consent. Adverse event reports were examined for possible moderate or severe hypersensitivity reactions, including anaphylaxis.

Anaphylaxis was assessed per National Institute of Allergy and Infectious Disease (NIAID) criteria. *Results:* In 255 patients treated with ecallantide for HAE attacks through 12 March 2009, 10 (3.9%) reported possible anaphylaxis. Ecallantide was administered IV in 5 cases, and SC in 5 cases. In the cases with IV ecallantide, the reaction occurred upon 1st dose (1 patient also had reactions after doses 2 and 4); all 5 patients were negative for anti-ecallantide antibodies. In the cases with SC ecallantide, none of the reactions were reported after the 1st dose; all 5 patients had seroconverted to anti-ecallantide antibodies. Common symptoms associated with these reactions included pruritus, nasal congestion, rhinorrhea, sneezing, flushing, throat irritation, shortness of breath, and nausea. Some patients also had chest discomfort, urticaria, wheezing, hypotension, dizziness, and abdominal pain; 1 patient reported transient loss of consciousness. All reactions occurred within 1 hour after dosing; all patients recovered without sequelae. Five patients (3 IV and 2 SC) underwent a rechallenge procedure; 2 (1 IV and 1 SC) experienced a repeat reaction and are ineligible for further dosing. The other 3 patients successfully completed the procedure; 1 patient received 1 additional dose and 1 received >16 additional doses with no further reactions. *Conclusion:* Hypersensitivity, including anaphylaxis, is a risk associated with ecallantide treatment for acute HAE attacks. Different mechanisms may have contributed to reactions following IV versus SC dosing. Antibody testing for ecallantide, skin tests, and rechallenge procedures may help assess the risk of future ecallantide doses.

Longitudinal analyses of herpesvirus loads in severe drug eruptions: persistently high Epstein-Barr virus loads in peripheral blood of Stevens-Johnson syndrome and toxic epidermal necrolysis patients

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Poster

Background: Evidence is accumulating that various herpesviruses reactivate during the course of drug-induced hypersensitivity syndrome (DIHS); however, no previous studies were extended beyond the acute stage. Thus, long term follow-up after clinical resolution of these herpesvirus reactivations in patients with DIHS and the involvement of these herpesviruses in other severe drug eruptions, Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) have not been evaluated. *Materials and Methods:* To elucidate the involvement of these herpesviruses during and after severe drug eruptions, Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6) and cytomegalovirus (CMV) DNA loads in peripheral leukocytes were determined using real time PCR from the onset to the long after clinical resolution of the diseases. Patients with SJS/TEN (n=19) and patients with DIHS (n=26) that visited our hospital were enrolled in this study. Diagnosis of SJS, TEN, and DIHS were made based on each criterion; patients with eczema/dermatitis (n=16) were enrolled as control group. Blood samples were obtained from the patients on or near the day of the initial presentation and the long time after the clinical resolution after withdrawal of immunosuppressive agents for the treatment (>501 days after onset); additional samples were subsequently obtained from the patients on a monthly basis. *Results:* High EBV DNA loads were detected in the patients with SJS/TEN throughout the observation period up to 4 years after resolution. EBV DNAs were detected regardless of their use of immunosuppressive agents. The mean value of EBV DNA loads at the acute stage in patients with SJS/TEN was significantly higher than that of patients with DIHS ($p<0.05$) or control group ($p<0.05$). In contrast, high HHV-6 DNA loads were detected in patients with DIHS during the acute stage but not in patients with SJS/TEN ($p<0.05$) or with control group ($p<0.05$). No significant difference was found between SJS/TEN and DIHS patients in CMV DNA loads. *Conclusion:* These results indicate that underlying viral infections are different between SJS/TEN and DIHS, and could cause a marked deviation in the pathological phenotype of severe drug eruptions. EBV might be a risk factor of SJS/TEN or possibly associated with pathogenesis of the disease.

Cross delayed reactivity between cyclophosphamide and chlorambucil

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Poster

Cross reactivity reactions between alkylant antineoplasic agents are exceptionally reported in the literature. We described a case of a 39-year-old man who was hospitalized for nephrotic syndrome due to an extramembraneous glomerulonephritis. This patient was treated according to Ponticelli-like protocol by prednisone (0.4 mg/kg), followed by 2 mg/kg of cyclophosphamide. Ten days after starting cyclophosphamide, he developed a generalized skin eruption with pruritic erythematous plaques. Cyclophosphamide was withdrawn and the skin eruption resolved within two weeks. Because of non remission of his nephrotic syndrome, a combined therapy of prednisone and chlorambucil (0.2 mg/kg) was started. On the Fifth day of this treatment, the patient noted a generalized skin eruption, similar to that observed with cyclophosphamide. The blood count and the biochemical profile obtained during the acute reaction were normal. A skin biopsy showed a perivascular infiltration with vacuolar interface dermatitis, compatible with toxidermia. Chlorambucil was discontinued with resolution of skin eruption. Eight weeks later, drug skin tests were perfomed on the patient (patch and intradermal tests to cyclophosphamide and patch test to chlorambucil) were positive to chlorambucil but not to cyclophosphamide. Subsequent therapy with tacrolimus was uneventful. Throughout this case, we describe a possible cross reactivity between cyclophosphamide and chlorambucil. To our knowledge, this is the first case reported for delayed-type reaction.

Analysis of clinical diversity of urticaria and angioedema induced by non-steroidal anti-inflammatory drugs (NSAIDs) in Japan

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Poster

Backgrounds: The pathogenesis of urticaria and angioedema induced by non-steroidal anti-inflammatory drugs (NSAIDs) is still obscure. Then, we analyzed the clinical characteristics of patients with

NSAIDs-induced urticaria and angioedema without asthma in Japan. *Methods:* We retrospectively collected the cases of NASAIDs-induced urticaria and angioedema from Japanese medical journals in 2000-2009. *Results:* Fifty-three patients, which have been reported to be caused by NSAIDs, were analyzed. The sex ratio was 2.1:1 as male: female, the mean age was 37 years old and the range was 5-76 years old. Among these 53 patients, the frequency of patients with chronic urticaria and those with allergic rhinitis and nasal polyp was 52.8% (28 patients) and 11.3% (6 patients), respectively. The mean total serum IgE value was 625.8 IU/ml and the range was 32-2500 IU/ml. In 49 of these patients, urticaria and/or angioedema were induced within 5 minutes to 14 hours by aspirin at a dose of 25-1000 mg. Clinical manifestations induced by NSAIDs were urticaria in 25 patients (51.0%), angioedema in 12 patients (24.5%), and the both in 12 patients (24.5%). Skin prick test was performed with aspirin in 13 patients, and the results were all negative. Leukotriene antagonists were effective in 2 of 4 patients administered but aggravated the symptoms in the others. Meloxicam of relatively selective cyclooxygenase-2 (COX-2) inhibitor was used safely in 4 of 6 patients administered but not in the others. Antihistaminics were effective in 3 of 4 patients administered but not in the other. *Discussion:* About half of the cases of NSAIDs-induced urticaria and angioedema without asthma were complicated with chronic urticaria and antihistaminics were usually effective to urticaria in these patients. On the other hand, leukotriene antagonists were generally effective to NSAIDs-induced asthma but not so much effective to urticaria and angioedema. Also, selective cyclooxygenase-2 (COX-2) inhibitor may be used safely in some cases with NSAIDs-induced urticaria and angioedema.

Drug allergy as a risk factor for a second drug allergy

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Poster

Background: Drug rash with eosinophilia and systemic symptoms (DRESS) syndrome is a severe hypersensitivity reaction which could also cause reactivation of viruses or new sensitization. *Case Report:* A case of a 51-years old male, who was receiving carbamazepine for one month after brain surgery is reported. The patient was treated because of infected postoperative pulmonary embolisms. Liver enzymes values were already high, which was attributed to side effects of carbamazepine. Therapy with low-molecular-weight heparin, warfarin, omeprazole and amoxicillin-clavulanate was initiated. On 10 day of therapy he developed diffuse erythematous rash, severe liver damage and peripheral eosinophilia (14%). Skin symptoms were at first attributed to amoxicillin which was withdrawn. 2 hours after receiving another dose of carbamazepine he developed high fever and intensive generalized rash. All drugs except warfarin were immediately withdrawn. Next day decline in liver enzymes value was observed; but increase of peripheral eosinophilia (up to 23%), generalized maculopapular rash on abdomen and lower extremities and rash with follicular accentuation was seen on the patient's face and neck. There was no lymphadenopathy, atypical lymphocytes in peripheral blood, urticaria, skin exfoliation, involvement of mucosa. The chest X-ray and kidney function were normal. Patient was treated with steroids in high dose He was discharged from the hospital 10 days later with no skin manifestation, mild eosinophilia (12%), and moderately elevated liver enzymes. Reactivation of HHV 6 was not confirmed. One month after reaction skin patch tests were positive with carbamazepine and amoxicillin. SIgE levels to penicillin G and V, amoxicillin were negative. Basophil activation test was positive to amoxicillin. Lymphocyte transformation test was positive to carbamazepine and amoxicillin. *Conclusion:* This patient developed DRESS 1 month after introduction of carbamazepine, and 10 days after introduction of amoxicillin. It has never been reported that amoxicillin could induce DRESS. Confirmed late and immediate hypersensitivity for amoxicillin could suggest that another sensibilization occurred during acute phase of DRESS. As amoxicillin was given into a highly activated immune system a flare up reaction probably occurred with persisting second sensitization. There are some case reports of such flare-up reactions, but as far as we know immediate hypersensitivity was not clearly confirmed until now.

Carboplatin desensitization – Effective alternative protocols in difficult cases

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Poster

Background: Carboplatin (CP) is an effective treatment for paediatric brain tumors. This drug has increasingly been reported to cause hypersensitivity reactions (HSR), most of them immunologically mediated. *Aim:* To describe a modified desensitization (DZT) protocol to CP in severe immediate reactions. *Methods and Results:* Based on previous own experience of successful DZT procedures with CP in children with brain tumors (17 DZTs), the authors applied the same protocol (M. Castells) in 3 new cases. Four children with 9 (A), 3 (B), 7 (C) and 3(D) years old with brain tumors had been treated with CP and vincristine (V) using a scheme based on SIOP protocol. After the induction phase, with V and CP and a pause of 3/4 weeks, CP and V were administered every 4 weeks for 1 year. One child (C) had completed a full treatment scheme 5 years before without any problem. Immediate HSR to CP appeared between the 6th and 10th cycle. Two children (A, B) had severe anaphylaxis independently of pre-treatment. At this point it was decided to stop conventional approach. Although HSR were severe, CP remained the best choice and DZT was decided. SPT to CP were performed, before DZT, with negative results. Specific IgE for CP and cisplatinum was determined (Phadia® Sweden). The 12th step DZT protocol was applied to children A, B and D. Patients A and B had severe anaphylaxis at the beginning of step 12 (A - total dose 59 mg; B - 70 mg). A 2nd DZT attempt was made, according to C. Cohen protocol. Similar reactions occurred. Because, in child B, sIgE was positive for CP and cisplatinum, the tumor was not removable and had a fatal prognosis, all treatments were stopped. In patient A, a 2 day, modified protocol from C. Cohen, beginning with 0.05 mg of CP was applied as the last DZT attempt, with successful results in 4 cycles. Patient D performed 1 DZT with M. Castell's protocol, with reaction. In alternative, C. Cohen was applied with good tolerance. As child C had previous severe reaction, C. Cohen protocol was performed with no reactions. *Results and Comments:* The authors have stated previously

that more experience, with the 12 step protocol, was needed in order to evaluate its efficacy and safety in children. Most severe reactions during DZT occurred in children with previous serious HSR. In the patient with a proved underling IgE mechanism both DZT protocols were unsuccessful. A 2 day DZT protocol was successfully applied to the patient with severe reactions to both previous protocols.

Hypersensitivity reactions to non-steroidal antiinflammatory drugs: patterns of responses

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Poster

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) are the most important group of medicaments involved in hypersensitivity drugs reactions. The mechanisms include IgE dependent responses, T cells mediated reactions and cross-intolerance, being this the most frequent. Within this, respiratory responses have been the most studied but cutaneous manifestations in absence of airways involvement also occur. Few studies with only or predominant skin involvement have been carried out. *Objective:* We describe a large group of 400 patients with skin manifestations accompanied or not by other organs involvement induced by cross-intolerance to NSAIDs. *Methods:* We performed an allergological study evaluating patients with suggestive symptoms of hypersensitivity reactions to one or several NSAIDs. Drug provocation test was carried out with an alternative NSAID to assess cross intolerance or not. Atopy status was assessed with a clinic questionnaire, a standard panel of inhalant allergens by skin-prick-test and total IgE measurement. *Results:* Urticaria and/or angioedema were the most common clinical entities reported. Most reactions occurred less than 1 hour after drug intake. The most frequent NSAID involved was a propionic acid derivative (ibuprofen). Patients with skin reactions to multiple NSAIDs showed an increased frequency of atopy ($p<0.0001$) compared to a control group that tolerated NSAIDs. *Conclusion:* According to these data, urticaria and angioedema induced by NSAIDs is a common problem that affects many subjects with hypersensitivity reactions to these drugs and can be diagnosed following a validated protocol. The most important drug was ibuprofen, what indicate the predominant role of this compound in hypersensitivity reactions to NSAIDs.

Tolerance to etoricoxib in patients with urticaria and/or angioedema with cross intolerance to non steroidal anti-inflammatory drugs (NSAIDs)

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Poster

Objective: NSAIDs are the most important anti-inflammatory agents. Within adverse reactions, the most frequent is hypersensitivity due to cross intolerance. The need to find a safe alternative drug is a frequent clinical problem. Previous studies have shown that COX-2-selective inhibitors (COX-2-SI) are usually well tolerated in these patients. The aim was to study tolerance to etoricoxib in patients with cross-intolerance with hypersensitivity reactions to NSAIDs including paracetamol. *Methods:* Patients with skin hypersensitivity reactions to NSAIDs were studied. Cross-intolerance was confirmed by clinical history and/or challenge. In those subjects reacting to paracetamol as well as those with tolerance, we assessed the response to etoricoxib by oral provocation. *Results:* We evaluated 268 patients with skin reactions and confirmed NSAIDs hypersensitivity. The 60% was female and 40±15,61 years old. Ibuprofen was the most frequent NSAID involved (56,7%), followed by ASA (50,7%) and metamizol (34,7%). The most frequent entity was urticaria (48,5%) and most of the reactions (46,7%) occurred less than 1hour after drug intake. A total of 52 patients responded to paracetamol. In this group, 13 patients (25%) presented symptoms after receiving etoricoxib. In a representative sample of 80 cases with good tolerance to paracetamol, etoricoxib was well tolerated. *Discussion:* Although most of studies attributed good tolerance to COX-2-selective inhibitors in patients with skin symptoms induced by multiple NSAIDs, the incidence for reactions found in our group was higher. A condition to find positive cases was a previous confirmed intolerance to paracetamol.

Delayed reactions to iodinated contrast media: two case reports including LTT studies

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Poster

Background: All iodinated contrast media (ICM) may cause both immediate (≤1 h) and non-immediate (>1 h) hypersensitivity reactions. Several investigators have found skin testing to be useful in confirming ICM allergy, especially in patients with non-immediate skin eruptions, but a negative skin test is not a guarantee against a future reaction. *Methods:* We report on two patients who presented maculopapular pruriginous generalized exanthema, 3 days and respectively 7 days after administration of ICM (Iohexol). The allergological study was carried out by performing skin testing (prick, intradermal and patch tests) with different ICM. However, as we were dealing with oncological patients who would further require investigations with ICM, Lymphocyte Transformation Tests (LTT) were also performed in the attempt to select a safe alternative. According to published data, LTT was considered positive if the stimulating index (SI) was > 4. *Results:* Prick and intradermal (ID) tests in immediate reading were negative to all ICM in both patients. Patient 1: ID skin tests resulted positive 24 hours after inoculation only for Iohexol and Iodixanol and the patch tests were all negative. LTT were positive to several ICM, including iobitridol and iomeprol (SI>4 at two concentrations), that had resulted negative both in ID and in patch tests. Patient 2: All ID tests were negative, but the epicutaneous tests were positive for Iomeprol, Iobitridol, Iodixanol, Iohexol. LTT was also positive to all these ICM but also to Amidotrizoate (SI>4 at two

concentrations), that had resulted negative in the skin tests. In both patients all the tests including LTT were negative to Gadoteric acid, that they posteriorly tolerated. The LTT with all the ICM at the same concentrations resulted negative in two healthy controls. *Conclusions:* The LTT proved to be a useful technique in these two cases, by allowing us to offer a safer alternative to these patients, while skin tests failed to identify all the possible cross-reactivities. Various available diagnostic tools should be used for better understanding of the specific immunological mechanisms involved, with consequent benefits for the patients when tolerability of related drugs is concerned.

Desensitization to rifampicin in non-immediate skin hypersensitivity reactions

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Poster

Background: Rifampicin (RF) is a semi-synthetic antibiotic, very efficient against mycobacteria, brucella and staphylococci. Adverse effects have been reported in up to 20% of patients most frequently flu like syndromes. Cutaneous reactions have been observed in 0.5-5% of patients. Rifampicin can elicit exanthema and urticaria but their pathomechanisms are not known. *Aim*: To report hypersensitivity reactions to RF in patients, referred to our Dug Allergy Unit, description of clinical approach, diagnostic procedures and treatment decision plan. *Methods:* We report 9 cases (age between 2 – 72 years) of cutaneous non immediate hypersensitivity reactions during treatment with RF. Six patients were HIV+ (4 also with Hep C) with associated pulmonary tuberculosis (PT), being treated with the classical 4 drug scheme for PT, including RF. The remaining patients were seriously hill: 2 with tuberculous meningitis and one septic patient with PT. All patients had isolated generalized exanthema, appearing from the 4th to the 14th day after beginning of treatment with RF. As all patients were critically hill and standardized diagnostic procedures were not possible at this point, all antimycobacterial agents were discontinued until the reaction resolved. Reactions were attributed to RF based on a detailed clinical history, and the re-introduction of each drug every 4 day with reappearance of clinical signs after RF (positive rechallenge therapeutic test). Since therapy with RF was the first line treatment, desensitization (DZT) was decided. In all but 2 patients, DZT was performed after complete remission of reactions. Two protocols were used based on the severity of the underlying disease and characteristics of reaction. In the 2 patients with meningeal tuberculosis and in the one with sepsis, a one day protocol was applied with success. A 3 day DZT protocol beginning with an initial dose of 2 mg was applied in 6 patients (seven procedures), adapted to achieve the therapeutic dose. Concerning the outcomes, one patient reacted after the 1 day protocol and tolerated the 3 day DZT. In one, both protocols failed at 100 mg. One out of ten DZTs was not accomplished by either procedures. *Comments:* DZT with RF has been reported, mostly for immediate reactions. In our 9 cases clinical tolerance, in non immediate reactions to RF was mostly achieved using a 3 day protocol The authors will speculate on the success of the 1 day protocol in more critically hill patients.

Drug hypersensitivity reactions in a paediatric population

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Poster

Background: Drug hypersensitivity reactions (HR) are less frequently reported in children and are usually associated with antibiotics (ATBs) and non-steroidal anti-inflammatory drugs (NSAIDs). Aims: To characterize the HR, namely suspected drugs, symptoms, and the result of the drug allergy study of the paediatric population referred to our hospital. *Patients and methods:* Retrospective study of the data collected from patients aged under 18 referred to our Drug Allergy Unit. *Results:* Seventy-three patients were studied, aged 1 to 17 years old (median – 10); 53.4%, 53% atopic. The patients reported a total of 112 HR; 37% patients reported more than 1 HR. Forty-eight HR were non-immediate. Skin symptoms occurred in 90.2%. Anaphylaxis was reported in 11/112 HR (9.8%) and the suspected drugs were ATBs in 7, NSAIDs in 2, deflazacort in 1 and 1 occurred during surgery; adrenaline was administered in 1. In 8 HR the child was admitted in the hospital due to its severity. ATBs alone were the suspected drug in 53/112 (47.3%) DHR [beta-lactams (BL) in 48, non beta-lactams (NBL) in 5], NSAIDs in 36/112 (32.1%) and other drug classes in 15; in 8 HR there was more than one drug involved. In the BL suspected reactions (n=53), amoxicillin/clavulanic acid was the suspected drug in 28 and cephalosporins in 10. Skin tests were positive in the study of 3 HR and specific IgE was positive in 2. Drug challenge with the suspected drug was performed in 34 and was positive in the study of 9 HRs. The diagnosis was confirmed in 14 (26.4%) and excluded in 26 (49.1%). In the NBL suspected reactions (n=6), macrolides were the suspected drug in 4 and co-trimoxazole in 2. The diagnosis was confirmed in 1 HR and excluded in 1. In the NSAIDs suspected reactions (n=43), the most common drug involved was ibuprofen (17). In 3 HRs, 2 NSAIDs were suspected concomitantly. The diagnosis was confirmed in 9 (20.9%) HR and excluded in 7 (16.3%). Three HR ocurred during the perianesthesia period. Drug challenge with the suspected drug was performed in 49.1% of the HRs. The diagnosis of drug hypersensitivity was confirmed in 25.9% and excluded in 33% of the HRs. *Conclusions:* ATB, namely BL, were the most common suspected drugs, followed by NSAIDs. Anaphylaxis was undertreated. In the BL suspected HR, diagnosis was confirmed or excluded in 3/4 of the HR, compared to 37.2% in the NSAIDs HR, possibly because drug challenge with the suspect drug was performed more frequently in the first (64.2% vs 37.2%).

Atypical clinical presentation of hypersensitivity reaction to penicillin in a child with Borreliosis

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Poster

Background: Antibiotic hypersensitivity reactions are a major health concern as they can be a significant cause of morbidity and mortality.

Diagnosis can be challenging and, in some cases, the lymphocyte transformation test (LTT) can be the only tool to confirm the diagnosis of drug hypersensitivity. *Case description:* We report the case of a 34 month-old girl that due to fever and odinophagia (interpreted as tonsillitis), was medicated with amoxicillin without improvement. Three days later she began cefaclor, with the same result. She was then given 2 shots of penicillin, with favourable evolution and apyrexia after 48h. Two weeks later, in the same places as penicillin shots, two nodular lesions appeared, with progressive worsening. Due to the severity of these lesions, she was admitted at her local Hospital. Shortly after she developed fever and was submitted to a surgical intervention at her buttocks for drainage of suspected abscess (not confirmed). Due to deterioration of her general status and suspected infectious panniculitis, she was transferred to a Central Hospital, and began flucloxacillin and clindamycin. Three days later, a disperse exanthema appeared and an Allergist was called. Flucloxacillin was stopped and the exanthema faded away, but a cutaneous erithema migrans, oedema of the left hand and both feet developed. The white blood cells differential showed the appearance of eosinophilia (3000/uL), and the aPT time was <20. Parvovirus B19, Epstein Barr virus and Borrelia IgM antibodies were positive, as well as Herpes 6 virus DNA. A skin biopsy showed dermis with a predominant lymphocytic inflammatory infiltrate and the presence of Borrelia burgdorferi DNA. Prednisolone (1.5 mg/kg/day) and azithromycin for Borreliosis (14 days) were initiated, with clinical improvement. The inflammatory lesions on both buttocks slowly disappeared. Specific IgEs to beta-lactams were negative and the immunophenotyping and lymphocite function in vitro study were normal. LTT was positive for Penicilin 100 ug/mL with a SI of 60.2. *Comments:* This child developed a delayed severe local reaction to penicillin, misinterpreted as panniculitis. The clinical history, with the time elapsed between shots and severe inflammatory lesions, pointed to the diagnosis of delayed hypersensensitivity reactions to penicillin. She was successfully treated for Borreliosis (her basic illness) with a non-beta-lactam antibiotic. The dignosis of beta-lactam allergy was confirmed by LTT.

Laboratory findings of the patients with advers skin reactions to hepatitis treatment

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Poster

Background: Interferon and Ribavirin are used in treatment of chronic hepatitis C. This type of treatment induces many adverse reactions, most of them involve skin (ADRS). *Aim of the study:* to evaluate the type of skin reaction and changes of laboratory analyses as eosinophils count, IgE level and liver enzymes induced hepatitis treatment. *Patients and Methods:* 19 patients (14 (73.7%) women), average age 51.0 [48.0-62.0] with ADRS to hepatitis B and C treatment were investigated in Vilnius University hospital "Santariskiu klinikos". 16 patients (84.2%) were treated with the combination of two drugs: Interferon and Ribavirin. 9 (47.4%) patients were treated with Interferon and 10 (52.6%) – with Pegilated interferon. A drug hypersensitivity questionnaire (ENDA questionnaires) was filled in and reaction types were evaluated. The laboratory results till and after start of advers drugs reactions was evaluated. *Results:* 9 (47.4%) patients had a maculopapular skin rash and it was the most frequent clinical manifestation. 4 (21.1 %) had an urticarial vasculitis and 2 (10.5%) patients had only a severe skin itching. Four others patients had one of skin eruption: there are injection site's skin infiltration, angioedema, hemorrhagic purpura and eczema. The average time of delay between the start of treatment and ADRS was 1.0 [0.24-4.0] month with minimum time at the first injection and maximum at the 6th month. All ADRS were non-immediate type with average time of delay 12.0 h [5.0-12.0 h]: minimum – 2 h and maximum – 96 h and it did not depend on clinical manifestation. Median level of blood eosinophilia was 0.045 x 109/l [0.0025-0.07 x 109/l] and elevated only in 4 patients. All ADRS are non-immediate type and IgE was not implicated in pathogenesis of reactions; the median level of IgE was 25.0 U/ml [19.2-67.6 U/ml]. The change of ALT and AST after ADRS rise was not significant. We have no differences between the ADRS and laboratory changes. *Conclusion:* We have not found any significant changes in eosinophils count, IgE level or liver enzymes for the patients with adverse skin reactions involved in hepatitis treatment.

Successful meropenem desensitization in a patient with multidrug-resistant *Acinetobacter pneumonia*

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Poster

Objective: To report a successful case of meropenem desensitization in a patient with multidrug-resistant ventilator-associated *Acinetobacter pneumonia*. *Case report:* A 4-year-old male patient with cerebral palsy and neuromotor retardation presented to our university hospital with symptoms of high fever (39-40°C), cough and shortness of breath. He was seen in emergency department and then admitted to our allergy and pediatric respiratory diseases department with the was diagnosis of aspiration pneumonia and total atelectasis of left lung. He had a history of meningitis at the age of 2 years and underwent ventriculo-peritoneal shunting because of post-meningitic hydrocephalus. He was using anticonvulsant drugs beacuse of epilepsy, and was undertreatment with levothyroxine because of hypothyroidism. He had antibiotic allergy, however the name of the responsible drug was not known. His treatment was started with cefotaxime (i.v). He developed respiratory failure 8 hours after and needed endotracheal intubation and mechanical ventilation. Because of continued high fever and worsening clinical manifestations meropenem was started instead of cefotaxime. On day 4, he developed urticarial rash, and antibiotic treatment was changed as clindamycin and amikacin. On day 25, *Stenophomonas maltophilia* was grown in tracheal aspirate culture which was only sensitive to meropenem-imipenem. Thus, desensitization with meropenem i.v was started. Increasing doses of meropenem was administered every 15 minutes beginning with 0.01 mg until one third of the daily dose (120 mg/kg/day) was achieved. On day 30, he had gastrointestinal bleeding and clinical findings continued to deteriorate, with overwhelming sepsis. Teicoplanin was added to treatment. Positive inotropic support for 6 days did not improve the clinical findings and hemodynamic derangement, and he

died on 36th day of admission. *Conclusion:* Antibiotic desensitization is a treatment option for patients with IgE-mediated antibiotic allergy when no other alternative exists for treatment of severe bacterial infections. In our patient meropenem treatment has been continued for 11 days without any reactions to meropenem following successful desensitization.

Lessons from two cases of anaphylaxis to proton pump inhibitors

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Poster

Background: Proton pump inhibitors (PPIs), which are widely used for the treatment of peptic ulcers and gastroesophageal diseases, reduce both basal and stimulated gastric acid secretion by inhibiting the parietal cell enzyme H(+)-K(+)-adenosine triphosphatase. To date, there have been several reports about hypersensitivity reactions to PPIs. *Materials and methods:* Here we present two patients with anaphylaxis to PPIs, one each to lansoprazole and omeprazole. We also suggest the lessons from these two cases. *Results:* A 74–year old woman visited the emergency room (ER) complaining of dizziness, dyspnea, and generalized skin rash after taking the orally disintegrating form of lansoprazole. Beginning 1 month earlier, she had taken esomeprazole and mosapride for gastroesophageal reflux disease without any side effects. She was diagnosed with an anaphylaxis to the orally disintegrating form of lansoprazole. A 49-year-old woman was referred to the allergy clinic for evaluation of her previous anaphylactic reactions. Two years earlier, she experienced anaphylaxis after taking clarithromycin, amoxicillin, and omeprazole for *H. pylori* eradication. At that time, she developed generalized urticaria, facial angioedema and dyspnea; a physician informed her that those reactions may be induced by penicillin. She experienced a second anaphylactic reaction after taking an aluminum-based antacid, itopride and omeprazole for dyspepsia. She was diagnosed with an anaphylaxis to omeprazole by oral provocation test. *Conclusion:* Physicians should be more prudent in prescribing PPIs owing to the possibility of hypersensitivity, and should explain the risk of anaphylaxis to their patients.

Mast cells involvement in a patient with infusion reaction to Rituximab

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Poster

Background: Infusion reactions (IR) are a subgroup of adverse drug reactions (ADR) against infused drugs, especially monoclonal antibodies. In preclinical studies, IR occurred in 15% patients following the first infusion of Rituximab and 5% in placebo patients. IR decreased to 2% following the second infusion in both groups. The presence of Human Anti-Chimeric Antibodies (HACA) may be associated with IR. A total of 96/1039 (9.2%) patients with rheumatoid arthritis tested positive for HACA following therapy with Rituximab. No specific immunoglobulin E against Rituximab have been reported so far. *Case report:* A 44-years-old female with erosive rheumatoid arthritis had undergone various therapeutic regimen including a.o. Etanercept, Adalimumab, Infliximab without complete remission. She received three doses of Rituximab in 2006. Another course was given at the end of 2007. She then presented with an acute flare of her rheumatoid arthritis in 10/08, despite continued co-medication with Methotrexate, Prednison, Diclofenac, Misoprostol and Hydroxychloroquin. She again received Rituximab in 10/08. 7 days later she reported a strong itch which disappeared completely without medication. Rituximab was given again 2 weeks later following the usual pre-medication with Paracetamol, Hydrocortison and Clemastin. After 55 minutes (200 mg/h) she developed urticaria and angioedema. Rituximab was stopped; Clemastin, Hydrocortisone and Adrenalin were given. Symptoms flared up again after resuming the infusion with 100 mg/h. 90 min after another dose of Clemastin i.v. the swelling resolved. *Results:* Tryptase was assessed during the initial flare and was increased to 17.4 µg/l (normal 13.5 µg/l), dropping to normal values one hour later. During the initial flare, CH50 was 20 U/ml (normal value 28-45 U/ml), C3 0.9 g/l (normal value 0.8-1.8 g/l), C4 0.14 g/l (normal value 0.10-0.40 g/l). Two weeks later all parameters were within normal ranges. Skin prick and intracutaneous (1:1) tests with Rituximab were negative two months later. In a retrospective analysis, levels of HACA were at their highest concentration at the time of IR. *Discussion:* We postulate following pathomechanism for the grade III anaphylactic reaction: IgG-mediated anaphylaxis with complement activation and consequent mast cell activation through complement cascade products or anaphylaxis through direct IgE-mediated mast cell activation. Both pathomechanisms would correspond to a type β ADR.

Drug-induced hypersensitivity syndrome and subsequent arthritis

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Poster

A 64-year-old Japanese woman with arrhythmia developed a fever and maculopapular rash over her face and trunk 4 weeks after the initiation of oral mexiletine. At first withdrawal of mexiletine and intravenous hydrocortisone did not improve her eruption, and she was hospitalized on 5 January 2009. Laboratory studies showed leukocytosis (15,900 /µl) with atypical lymphocytes, and liver dysfunction (AST 100 U/L, ALT 182 U/L). On the 14th day of hospitalization, polymerase chain reaction detected the HHV-6 DNA (3.4x102 copies in 1 µl of whole blood), but no other herpes viruses. A skin biopsy demonstrated lymphocytic infiltration in the epidermal-dermal junction and liquefaction degeneration of the basal cell layer. Patch test and drug-induced lymphocyte stimulation test demonstrated positive reaction to mexiletine. We diagnosed her with mexiletine-induced hypersensitivity syndrome. Oral prednisolone at 40 mg/day was effective in improving skin eruption, fever, and liver dysfunction, and prednisolone was weaned carefully. The bilateral wrists and knees were slightly swollen with pain after prednisolone dose was reduced from 20 to 15 mg/day on 5 March. Serum MMP-3 was increased to 582 ng/ml on 27 May, which was in the normal range 17.8 ng/ml on 13 January. This is the first case of DIHS associated

with arthritis, which may result from immune system dysregulation by drug allergy and/or HHV-6 reactivation.

Late cutaneous reaction to iodixanol

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Poster

Background: Since their introduction, iodinated contrast media (ICM) have been among the most commonly used drugs. Adverse effects from the IV administration of ICM are generally mild and self-limited. Nonetheless, severe or life-threatening reactions can occur. The pathogenesis of such adverse reactions probably involves direct cellular effects, enzyme induction and activation of the complement, fibrinolytic, kinin and other systems. *Results:* The authors report a case of 67-year-old Caucasian woman with no history of previous allergic reactions that developed a maculopapular erythematous pruritic rash 72 hours after been submitted to a contrasted renal CT scan with iodixanol. She was attended at the emergency department and treated with parental corticosteroid and antihistamine and discharged with a domiciliary tapering treatment of oral prednisolone, loratadine and hidroxyzine. Twenty-four hours later the patient returned to the emergency department presenting generalized maculopapular rash and violaceous lesions on her legs, suggesting vasculitis. Laboratory studies revealed leucocytosis with neutrophilia and mild eosinophilia. No abnormalities on blood chemistry or coagulation were found. She was discharged maintaining oral corticosteroid and antihistamine and referred to our Drug Allergy Unit. All available ICM (iopromide, iomeprol, ioversol, iobitridol, iodixanol and meglumine ioxithalamate) were tested by skin prick (undiluted), intradermal (1/10 dilution) and patch tests (1/10 and undiluted). Immediate readings of skin prick (SPT) and intradermal (IDT) tests were negative. SPT, IDT and patch tests with iodixanol and IDT with iomeprol were positive at 48 and 72 hours. The patient was advised to avoid iodixanol and iomeprol and alternative ICM were suggested (iopromide and iobitridol). *Conclusion:* Although the macroscopic characteristics of the lesions strongly suggested a cutaneous vasculitis there is no histologic diagnosis because skin biopsy was not performed before starting corticosteroid treatment and when she attended our Drug Allergy Unit, the lesions had already resolved. We emphasize that a patient who is suspected to suffer from vasculitis should be promptly referred to a specialized institution for further diagnostic work up and management enabling invasive procedures such as biopsies and then iniatiation of appropriate treatment. We discuss the usefulness of skin tests in this type of reaction.

Hypersensitivity to ribavirin

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Poster

Background: Combined treatment with pegylated interferon (PEG-IFN) and ribavirin is currently recommended for the treatment of chronic hepatitis C virus infection. Many side effects related to this treatment have been reported and sometimes are so severe that they may lead to the transient or definitive interruption of therapy. Rare cases of hypersensitivity reactions have been described. *Results:* We report the case of a 45-year-old Caucasian woman, with a history of chronic hepatitis C, who started treatment with PEG-IFN alfa 2a plus ribavirin. Four months after starting the medication, she developed generalized pruritus and erythematous maculopapular lesions. The lesions disappeared spontaneously within 15 days after drug cessation. Once she had indication to re-establish therapy, she was referred to our Drug Allergy Unit. Skin prick (360 microg/ml), intradermal (1/1000 to 1/10 dilutions) and patch tests (1/1000 to 1/1 dilutions) with PEG-IFN alfa 2a were negative (immediate and late readings). Patch tests with different concentrations of ribavirin (10%, 30% and 50% in vaseline) were also negative. Subcutaneous challenge with a cumulative dose of 180microg of PEG IFN-alfa 2a did not cause any immediate or late reaction. Oral provocation challenge with 1000 mg of ribavirin did not cause any immediate reaction. Six hours after drug challenge, the patient developed generalized pruritus and erythematous micropapular lesions, which improved with oral antihistamine and prednisolone. She was advised to avoid ribavirin and monotherapy with PEG-IFN alfa 2a was suggested, but according to her Gastroenterologist currently the patient has no indication for that treatment. *Conclusion:* In the management of hipersensitivity reactions to combined therapy, the involvement of IFN and ribavirin is usually suspected when there is a rapid resolution after drug cessation. Skin tests with those drugs are seldom performed, as they are mostly unhelpful. Althougth hipersensivity reactions are frequently atributed to IFN, we emphasize the need of drug provocation challenge with both drugs because the culprit drug can be ribavirin, as in this case.

Kounis syndrome induced by piperacillin/tazobctam infusion

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Poster

The Kounis Syndrome (KS) is the concurrence of an acute coronary event with condition associated with a massive mast –cell degranulation, usually an anaphylactic or ananaphylactoid reaction (1) and it should be due to the inflammatory mediators, such as histamine, chemokines and leukotrienes released by mast cells (2), which are able to induce a coronary vasospasm in some patients (1, 2). KS was described firstly in 1991 by Kounis and Zafras as allergic angina or an allergic myocardial infarction (3). KS can be induced by Hymenoptera stings, food allergy and of course various drugs as well as contrast media, intravenous anaesthetics, NSAIDs and, of course, antibiotics (1). Beta-lactams are the commonest cause of KS among the antibiotics (4). We described a KS in a 62 y.o man induced by tazobactam/piperacillin administration. Case Report A 62 y.o obese man was admitted to the 4th Division of Pneumological Hospital A Galateo (Lecce). He referred cough, malaise and slight chest constriction since some days, with no fever or dyspnea. The Chest X-Ray confirmed the presence of a pneumonic process on the basal right lung. Electrocar-

diography showed a normal frequency with sinus rhythm White Blood cell count was higher (127.00/mm^3), blood pressure was 80/130 mmHg and haemogasanalytical values were satisfactory as well as pulse oxymetry (SpO_2: 97-98%). He didn't referred any drug allergy, despite he had been admitted twice in the General Hospital Vito Fazzi, firstly in 2002 at the Cardiac Surgery Unity for the partial rupture of a misdiagnosed thoracic aorta aneurysm and then in 2005 at the Thoracic Surgery Department for a spontaneous pneumothorax. We prescribed him theophylline, corticosteroids, diuretics and Tazobactam/Ampicilline (T/A) 4,5 g bis in die intravenously. Few minutes after the administration of T/A an erythematous generalized eruption appeared on the trunk and on the face as well as the tachycardia (approximately 140/bpm) while the pulse-oxymetry decreased at 78-79%. Antibiotic administration was promptly stopped, but dyspnea and tremors appeared too, associated with a generalized urticarial rash. Blood pressure collapsed to 70/40 mmHg. Patient referred intense chest and abdominal pains and his face showed high sufferance. One gram of hydrocortisone succinate was injected intravenously, through the venous peripheral catheter positioned during the admittance, followed by chlorpheniramine maleate diluted in 100 ml of saline solution and high-flow oxygen. Patient still referred a strong thoracic pain and an the urgently performed ECG showed a tachycardia with some extrasystoles and a ST segment depression in leads V4-V5-V6, suggesting an acute ischemic injury. Taking advantage by a slight increase of blood pressure (60/90 mmHg) and by the presence of the cardiologist, an other gram of Hydrocortisone succinate was injected to the patient associated to a slow infusion of nitrates. As soon as chest pain faded, administration of the latter was promptly interrupted. No epinephrine was administered. Cardiac enzymes were dosed during the acute episode and 6 hours later (Table 1). The patient improved and pulse-oxymetry demonstrated an oxygen saturation of 92%. The day after on a bood sample total IgE have bee dosed, showing a normal range of 54 IU/ml, but at a second sample 3 days later total IgE showed a boosted response with an increased range of 556 IU/ml (Phadia Company – Sweden). The specific IgE to Penicilloyl G, Penicilloyl V, Amoxycilloyl were not demonstrable by CAP (Phadia, Sweden). On the contrary, IgE to Ampicilloyl was present (0,23 kUA/l – normal range< 0,1 kUA/lt Phadia Company). By examining documentations of the preceding hospitalizations, we discovered patient had already assumed Sulbactam/Ampicillin in 2002 and Tazobactam/Piperacillin in 2005. Since there was no elevations of Penicilloyl G, Penicilloyl V, Amoxycilloyl and Ampicilloyl at a secondary control 3 days later, but there have been an increase of total IgE demostrating the specific immune response, probably towards the side-chain of Piperacillin (5), we concluded patient had got an IgE mediated anaphylaxis. For the peculiarity of symptoms and the risk of develop another KS, no skin tests were performed in such patient. As far as epinephrine is concerned, its use is quite controversial in Kounis syndrome for the possibility it increases oxygen consumption by cardiac muscle and favours arrythmia onset. Patients undergone to a cardiac intervention have a greater risk to develop a KS, especially if coronary stents have been positioned (6)

Table 1

Cardiac enzymes	0	6 hours later	9 days later
LDH (v.n. 266 – 500)	599 U/l	712	462
Creatinphosphokinase	145 U/l	404	51
CPK (v.n. < 180)			
Creatinkinase-MBi	4 ng/ml	7.63	2.2
CK-MB (v.n.< 5)			
Myoglobin (v.n. < 72 ng/ml)	211 ng/ml	414	68
Troponin-I (v.n. < 0.10)	< 0,10 ng/ml	<0,10	

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Cutaneous adverse drug reactions due to delayed sensitization to carboxymethylcellulose

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Poster

Background: Immediate hypersensitivity (HS) due to carboxymethylcellulose (CMC) is well known, 3 cases of delayed HS emphasize that this excipient can also induce delayed systemic symptoms. *Observations:* case 1: a 24 y.o. man had developed in 1996 a DRESS due to Tegretol® (carbamazepine and CMC), with positive patch tests (PT) done with carbamazepine at 10% in petrolatum. The following years, he had some onset of eczematous rashes following the intake of pills containing paracetamol or NSAIDs with negative IDT with these chemical classes. Case 2: a 48 y.o. with a history of chronic rheumatism and of photosensitization to ketoprofen, developed a maculopapular rash (MPR) 3 days after the injection of betamethasone, then a MPR of the trunk 24h after taking pills with piroxicam, the 2 drugs containing CMC. Case 3: a woman with a history of hypothyroidy and contact allergy to a wound dressing containing CMC for leg ulcers due to a protein S deficiency, had a pressure urticaria but also a more recent chronic generalized urticaria. Even in avoiding pressure, the generalized urticaria persisted, but it disappeared when Levothyrox® containing CMC was suppressed. The 3 patients had PT and when negative, also prick tests (Pt), IDT or oral provocation test (OPT) with CMC. The results were the following: PT all negative, delayed Pt (1+/3), positive IDT (1+/2) and 1 positive OPT. Skin tests and OPT with hydroxypropylcellulose were negative. *Discussion:* The CMC (also called carmellose, croscarmellose, E466) can induce

immediate but also delayed hypersensitivity. From our results, in delayed reactions to CMC, PT, Pt and IDT (1 mg/ml) can have positive results. If the latter are not observed, OPT with CMC can be conducted under hospital surveillance. In immediate reactions to CMC contained in injectable drugs it was reported that the oral administration of CMC was well supported due to its weak absorption. We emphasize that in delayed HS to CMC, there is no oral tolerance. There is no cross reactions with hydroxypropylcellulose.

Oral desensitization to allopurinol in a patient with tophaceous gout

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Poster

Background: We present an effective and safe oral desensitization to allopurinol in a kidney transplant patient with gout. A major problem to the treatment of hyperuricemia in patients allergic to allopurinol is the limited availability of suitable, equally effective, alternative, urate-lowering drugs. Conventional uricosuric drugs (probenecid and sulfinpyrazone) are recommended for allopurinol-intolerant patients with gout and "underexcretion" hyperuricemia who have normal renal function and no history of nephrolithiasis. Therapeutic options in those in whom traditional uricosuric drugs are contraindicated, ineffective, or poorly tolerated include slow oral desensitization to allopurinol. This method is useful particularly in those who have failed other treatment modalities. *Materials and methods:* Department of Rheumatology contacted us because of a 66-year-old female with hypertension, chronic kidney disease and tophaceous gout who needed allopurinol to reduce hyperuricemia. The patient showed pruritic maculopapular eruption ten minutes after the second dose of allopurinol, without breath or angioedema. Skin reaction disappeared in hours with antihistamines. Cutaneous tests were negative. Oral controlled exposure to allopurinol was positive, reproducing the same symptoms. Therefore, we programmed a ten-day in-patient oral desensitization to allopurinol

Oral desensitization to allopurinol

1º Day	1 mg/5 ml	0.05 mg, 0.1 mg , 0.2 mg, 0.5 mg, 1 mg
2º Day	1 mg/5 ml	5 mg
	10 mg/5 ml	10 mg
3º Day	10 mg/5 ml	25 mg, 50 mg
4º-5º-6º Day	10 mg/5 ml	100 mg
7º-8º-9º Day	10 mg/5 ml	150 mg
10º Day	10 mg/5 ml	300 mg

Results and conclusion: In this case, the procedure was successful; however, therapeutic dose was achieved two days later because of minor manifestations. Cutaneous reactions can appear during and after desensitization and can be solved by dosages adjusted or cleaning period. Our patient continued therapy with allopurinol, and normal serum uric acid levels were reached and mantained. Severe allopurinol hypersensitivity reactions are not necessarily dose-dependent and do not always correlate with serum oxypurinol levels. The use of allopurinol only for accepted indications and in dosages adjusted for a patient's renal function may be the only means of minimizing the incidence of allopurinol hypersensitivity syndrome.

Itraconazole allergy

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Poster

Background: Allergic reactions to antifungal drugs are rare. Itraconazole has been rarely associated to cutaneous reactions, such as photosensitivity reactions, acute generalized exanthematic pustulosis, urticaria or angioedema. *Case report:* Female patient, 41 years-old, with a maculopapular pruriginous rash, developed at the 10th day of treatment with itraconazole (200 mg daily) for onichomicosis, initially located to the neck with ulterior generalization and development of angioedema of the lower lip. Since she was in the last day of treatment, itraconazole was stopped and not replaced. The patient had previously tolerated this drug, one year before. Laboratory evaluation showed leukocytosis (20900/μL; 85% neutrophils) and CRP 2.07 mg/dL. Itraconazole patch tests (10 and 30% in petrolatum) were negative. Lymphoblastic Transformation Test (LTT) with itraconazole was positive. The patient did not consent a challenge test with Itraconazole. She was treated with oral antihistamines and steroids, with favourable evolution and complete resolution in 4 weeks. *Conclusion:* Considering the clinical history, LTT results and since no other cause was identified, we considered itraconazole the responsible for the cutaneous lesions.

Hypersesitivity to non-steroidal anti-inflammatory drugs which turned out into contact dermatitis. Role of medical history taking

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Poster

On the base of past medical history of the patient directed as suspicion of non steroidal anti-inflammatory drug hypersensitivity we would like to emphasize the role of history taking in dealing with patients with drug hypersensitivity. We would like to show problems with past medical history receiving both from patients and from medical records. 65- years old patient, was referred to our department because of two incidences of angioedema in course of ketoprofene taking. He was referred to hip alloplasty but besides of 50 pack-years , hip pains and angioedema episodes his medical history was naive. Both episodes took place with ketoprofene taking but also with metalworking with arc machining. Type of symptoms coexistent with angioedema indicated also contact dermatitis. We confirmed moderate COPD, contact allergy to nickel. Drug hypersensitivity was ruled out by negative oral aspirin provocation test. Among 30 cases referred to department as drug hypersensitivity only three of them had clear history of reaction to one drug with all data available from patient. In the rest cases patients reported multidrug- connected reactions with lack of data necessary dignostics decisions. Copy of medical records are mostly inaccessible or do not contain description of symptoms. The poster indicates on clinical problem of multidrug related reactions and their proper diagnostics assignment.

Ertapenem-induced DRESS syndrome in a female patient

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Poster

Introduction: DRESS syndrome (drug rash with eosinophilia and systemic symptoms) is a life-threatening multi-organ system hypersensitivity reaction characterized by rash, fever, lymphadenopathy, hepatitis, and leukocytosis with eosinophilia. Ertapenem, a relatively new carbapenem antibiotic, has never been reported to cause DRESS syndrome. *Case report:* 44 year old previously healthy woman was hospitalized for a left frontal brain abscess. Patient had a remote history of penicillin and sulfa antibiotics causing hives. Brain biopsy was sterile, but the patient had previously been treated with azithromycin for presumed sinusitis. She was empirically treated with IV Vancomycin and Ertapenem. Two weeks later the patient developed DRESS syndrome. Antibiotics were discontinued and her symptoms of DRESS syndrome resolved. While on a drug holiday, the patient was re-challenged to Ertapenem and within one day developed DRESS syndrome. *Conclusions:* This is the first reported case of Ertapenem causing DRESS syndrome. A high index of suspicion is necessary to avoid missing this potentially fatal disease.

Hydroxyzine pamoate - induced acute generalized exantheatous pustulosis (AGEP) followed by psoriasis vulgaris

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Poster

Backgrounds: Both of acute generalized exanthematous pustulosis (AGEP) and psoriasis vulgaris are neurotrophilic inflammatory skin disease but the relationship between them have not been clearly understood yet. We have experienced a case of AGEP due to hydroxyzine pamoate (HP), which. was followed by development of psoriasis vulgaris in spite of no history of the pre-existing psoriasis. *Methods:* In order to examine cytokine profile in our case with AGEP followed by psoriasis, many kinds of cytokines in the serum were measured by Bio Plex cytokine assay kit. *Results:* Case report: An 81-year-old man was referred for generalized erythema with high fever to our hospital. He had taken an antihistamine, HP for 7 days and next day he developed itchy eruption on his back, chest, and limbs. The eruption had spread gradually on his whole body during next 8 days. On admission, he had desquamative erythema and red papules on his whole body associated with many aseptic pustules. The eruption was associated with high fever, and lumbar and finger pain. Laboratory examination showed leukocytosis of 18400/mm^3 and neutrophilia of 81.4%, and elevated level of C-reactive protein of 10.97 mg/dl. Histology of a skin biopsy specimen from his abdomen showed a subcorneal microabscess with neutrophils. No findings of vasculitis were found. He was treated with amoxicillin 750 mg/day, and his eruption and other symptoms were disappeared in 10 days. Patch testing with HP showed a positive result with erythema and pustular formation. Thus he was diagnosed as AGEP caused by HP. However, thirty days after his recovery, he developed psoriasis vulgaris, which continued for 1 year until now. Cytokines in serum were measured at some points during the clinical course. Many cytokine levels including Il-6, IL-8, IL-12, IL-17, TNF-α, interferon-γ and GM-CSF rose at the time of admission for AGEP. Conclusion: It is thougt that HP-induced AGEP itself might be involeved in the development of psoriasis though an elevation of cytokines including IL-8 and IL-17, which are key cytokines for psoriasis.

Aspirin re-desensitization: a report of two cases

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Poster

Aspirin desensitization is effective for treatment of the upper and lower airways in aspirin exacerbated respiratory diseases (AERD). After completion the desensitization protocol, patient must take aspirin daily. The intervals between aspirin intake should not be exceed more than 48 hours due to the risk of loosing desensitized status. We present her two cases with AERD requiring aspirin re-desensitization which are not seen in daily clinical practice. . Both cases were female and needed knee surgery. Withheld daily aspirin was ordered by orthopedic surgeon. The first cases readmitted to our clinic 15 days after surgery. Her first desensitization was performed almost two years ago and she was on 300 mg aspirin daily till the knee surgery. At first desensitization she had experienced only nasal reaction. Re-desensitization was performed in two days. Fist day, 20-40-80 mg aspirin was given at 90 minutes intervals. Eight hours after the last dose, she experienced severe rhinitis and relieved with nasal steroid, antihistamine. Second day, 150 and 300 mg aspirin was given 3 hours interval and observed only mild rhinitis. She is on 300 mg aspirin daily. The second cases readmitted to our clinic just before the knee surgery and re-desensitization was planned 24 hours after the surgery. The gap was 4 days. Eighty and 300 mg aspirin was given 3 hours intervals and 30 minutes after last dose she had only mild rhinitis. She is on daily 600 mg aspirin daily. Aspirin re-desensitization was completed in 1 and 2 days without severe reaction.

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