Investigation of the utility of *in vivo* and *in vitro* diagnostic procedures in correlation with clinical manifestation in drug hypersensitivity reactions

Summary of the Ph.D. Thesis

Katinka Ónodi-Nagy M.D.

Supervisor: Zsuzsanna Bata-Csörgő M.D., Ph.D., D.Sc.

Department of Dermatology and Allergology
Albert Szent-Györgyi Medical School, University of Szeged

Dermatology
Doctoral School of Clinical Medicine
Albert Szent-Györgyi Medical School, University of Szeged

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II. **Ónodi-Nagy K.**, Bata-Csörgő Zs., Varga E., Kemény L., Kinyó A. Antibiotic Induced Cutaneous Rash in Infectious Mononucleosis: *Overview of the Literature. Journal of Allergy and Therapy.* 2015; 6 (5).

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1. Introduction

Drug hypersensitivity

An adverse drug reaction is an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, alteration of the dosage regimen, or withdrawal of the product. Drug hypersensitivity reactions' incidence account for about 6 to 10 percent of all adverse drug reactions. The term "drug allergy" refers to a specific immune response to a medication acting as a hapten, the hapten-carrier complex functions as an allergen. The term drug hypersensitivity, besides allergy, includes also reactions of immune or inflammatory cells, a drug-dependent but not necessarily antigen-dependent stimulation of immune competent cells like T-cells and/or inflammatory cells by drugs. Direct drug interactions with immune receptors, like HLA, TCR (pharmacological interaction with immune receptors concept), or interactions with enzymes or receptors of inflammatory cells can also lead to drug hypersensitivity reactions.

The mechanisms underlying the development of a hypersensitivity reaction are complex, so the clinical picture shows heterogeneity. Drug hypersensitivity can be divided into groups by a combined approach, based on the time of symptom appearance, possible mode of action of the medication on immune and/or inflammatory cells, and immunologic mechanism. Gell and Coombs's classification links the clinical phenotype to the immune mechanism. This classification is important to devise testing strategies with the implicated drug. The immune reactions can be mixed, the character of a hypersensitivity reaction depends on the dominant immune mechanism.

Diagnostic procedure

The suspected underlying mechanism should be taken into consideration during the allergological work-up. If the clinical history and manifestation suggest the presence of drug hypersensitivity, *in vitro* and *in vivo* diagnostic tests can be performed.

In the *in vivo* methodology the first choice is skin testing; prick, intradermal and patch testing. In cases of severe reactions, a careful risk-to-benefit analysis must be undertaken if testing is decided, safety precautions and very high starting dilutions should be applied. If the available

information suggests that the probability of a hypersensitivity reaction is low, clinical history is unreliable, previous *in vitro* and *in vivo* tests did not lead to a conclusive result, a drug's metabolite or genetic disposition can be responsible for the symptoms, a drug challenge test may be performed.

Mast cell and basophil cell activation have a major role during an immediate, IgE antibody-mediated adverse reaction. Detection of drug-related IgE antibodies, basophil activation test, and detection of chemically varied liberated mediators, such as tryptase enzymes, histamine, and leukotrienes could be valuable *in vitro* diagnostic aids in immediate hypersensitivity.

Investigation and characterization of effector cells and corresponding inflammatory mediators are important in non-immediate hypersensitivity. *In vitro* tests are based on reproducing T cell activation *in vitro* and measuring inflammatory and cytotoxic mediator release by different assays. Searching for genetic markers may prove helpful in diagnostics.

Lymphocyte Transformation Test

Lymphocyte Transformation Test (LTT) is often considered to be the only reliable *in vitro* test to detect drug-sensitized T-cells and identify the culprit drug. However, more investigation is needed to improve its sensitivity and specificity with different clinical symptoms and drugs. According to the literature its calculated general sensitivity is 56.1%, specificity is 93.9%. The test is based on the detection of lymphocyte proliferation upon stimulation with the drug.

In our previous work, in my thesis at the Department of Dermatology and Allergology, University of Szeged, we investigated the reliability of LTT in the diagnosis of drug hypersensitivity. We studied which drugs were associated with a higher frequency in eliciting hypersensitivity reactions. Between January 2005 and January 2007, at the Department of Dermatology and Allergology, University of Szeged in Hungary, 4754 lymphocyte transformation tests were performed with 438 different drugs. This number was 4964 between January 2009 and January 2011, including 783 different drugs (230 new drugs compared to the previous period). The number of tests performed with penicillin, metamizole sodium monohydrate, acetylsalicylic acid, and diclofenac were equally high in both periods.

Among patients who had relatively reliable clinical data, we compared the positive LTT results with the negative ones for the most frequently suspected drugs and correlated the clinical manifestations with the positive LTT results for the different drugs. The highest number of

positive LTT results were detected in patients with maculopapular exanthems for various drugs. In our patients, the low percentage (10%) of positive LTT results originated from the relatively low reliability of the clinical data. It does strongly emphasize the importance of the drug history and the proper clinical diagnosis in this group of patients.

We correlated our patients' data on the reliable clinical history and recognized drug hypersensitivity symptoms to our LTT results evaluated in 2010. Clinical manifestations were angioedema, anaphylaxis, urticaria, vasculitis, maculopapular exanthema, granulomatous reaction, and fixed drug eruption. Metamizole sodium monohydrate, diclofenac, acetylsalicylic acid, penicillin antibiotics, allopurinol, ibuprofen, metoprolol, ramipril, perindopril, alprazolam, and lidocaine drugs were investigated. Data demonstrated the potential usefulness of identifying the suspected allergen. We concluded that false positive results are rare, although the overall negative predictive value and sensitivity seemed to be relatively low. We suggest that the clinical history and manifestation are crucial in the proper interpretation of LTT results.

Risk factors: infectious mononucleosis and hyperuricemia

Infectious mononucleosis is a well-known acute disease mostly caused by a widespread human γ-herpes virus, the Epstein-Barr virus (EBV), or a human β-herpes virus, the cytomegalovirus (CMV). The primary infection appears more often in children, adolescents, and young adults. Skin eruptions may develop during the infection. These eruptions are maculopapular exanthems, morbilliform eruptions which can appear on the entire body, and in severe cases the progressive skin reaction turns into erythroderma. A severe cutaneous reaction such as erythema multiforme is an exceedingly rare, although possible manifestation. The skin symptoms may develop due to the viral infection, however, these patients often use antibiotics and it is also well-known that viral infections enhance the risk of drug allergic reactions.

Eosinophil-rich maculopapular exanthems that occur in mononucleosis associated rashes are considered to be delayed-type hypersensitivity reactions, in which Th2 T cells are activated and secrete IL-4, IL-5, and IL-13 that lead to eosinophilic inflammation. However, the secretion of IgE and IgG4 by B-cells accompanies the reaction, connecting the delayed reaction to an immediate one.

Uric acid is a final product of the metabolic breakdown of purines in humans and is excreted normally in the urine daily. The phenomenon is termed hyperuricemia if the hydrogen urate ion

concentration in human blood plasma is above the normal range. Gout is a common form of inflammatory arthritis. Serum urate testing is remarkably useful, although hyperuricemia alone is not sufficient for the diagnosis of gout. There is evidence that hyperuricemia may, not independently, modestly increase the risk for coronary heart disease. Other investigations found hyperuricemia to be an independent risk factor of coronary and heart diseases, heart failure, stroke, and cardiovascular death. Studies have shown the potential cardioprotective effect of allopurinol therapy in decreasing the occurrence of acute cardiovascular events in gout and diabetes. Allopurinol, a drug for inhibiting urate production, is widely used. Use of the drug became prominent by the year 2005, in line with the investigation of its cardioprotective effect. The excessive drug prescription, around 2009 in Hungary according to our data, for conditions associated with urate excess, increased the appearance of allopurinol-induced hypersensitivity reactions, and severe cutaneous adverse reactions. The drug and its active metabolite, oxypurinol is considered to be responsible for its effects and subsequent adverse reactions. High starting dose, age, and comorbidities, like renal impairment, concomitant use of diuretics, or the presence of the HLA-B*B58:01 allele, can affect the development of allopurinol-induced cutaneous adverse reactions. The cutaneous and systemic symptoms, with potential morbidity and mortality, appear after a few weeks of allopurinol therapy. Relapses may occur even after the discontinuation of the drug.

2. Aims

I. We aimed to investigate the cutaneous reactions following amoxicillin treatment within infectious mononucleosis, to examine whether lasting drug-specific sensitization to penicillin or other antibiotics developed among these conditions.

II. We aimed to investigate the clinical and histopathological characteristics of allopurinol-induced adverse drug reactions to achieve a proper diagnosis early and sufficient information on probable future prevention.

3. Materials and methods

Study I: Infectious mononucleosis and drug hypersensitivity

Patient selection

At the Department of Dermatology and Allergology, University of Szeged in Hungary, among those patients who were treated between 2002 and 2012, ten young adults (5 men and 5 women, mean age 22.9, range 15-35 years) with a diagnosis of infectious mononucleosis, confirmed by EBV serological assay (specific IgM and IgG antibodies), associated with generalized maculopapular eruptions were examined for sensitization to antibiotics. Each of these patients had received antibiotic therapy prior to the appearance of skin eruptions. In all cases, the antibiotic was amoxicillin/clavulanic acid, in 2 cases in addition to penicillin the patients were given clarithromycin or cefixime as well. Given the clinical symptoms, the differential diagnosis should include Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS). Although clinically the skin symptoms can be indistinguishable the diagnosis of DRESS has quite strict criteria, which were not met in our patients.

In vitro tests: lymphocyte transformation test (LTT)

We examined 10 mononucleosis infectiosa patients with a history of penicillin intake, with *in vitro* method 1-1.5 months after the cessation of skin eruptions. This type of *in vitro* investigation is designed to determine the T-cell proliferation which occurs if there is sensitization to the drug. The lymphocyte transformation test was performed as described elsewhere with minor modifications. Briefly, peripheral blood mononuclear cells were isolated from heparinized peripheral blood and cultured under defined conditions with various concentrations of the suspected drugs (100 μg/ml and 10 μg/ml dilutions), in our cases with amoxicillin, amoxicillin/clavulanic acid, penicillin, and cefixime. We evaluated cell growth in the cultures. Cell growth was measured by using a colorimetric assay and an automatic microplate scanning spectrophotometer. The assay depends on the reduction of tetrazolium salt (MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by living cells, to form a blue insoluble formazan product. During the *in vitro* investigation, we used the spontaneous cell growth as a negative control, while the phytohaemagglutinin-stimulated cell culture served

as a positive control. The results were recognized as positive if the drug-stimulated cell numbers were at least twice higher that the negative control's (stimulation index > 2).

***In vivo* test: skin tests**

We performed *in vivo* cutaneous tests using penicillin in 6 patients with negative LTT to amoxicillin. The remaining 4 patients refused to consent to the test. Prick, intradermal, and patch tests were performed using penicillin's main antigens: major determinant benzylpenicilloyl poly-L-lysine (PPL), minor determinant mix sodium benzylpenicillin, benzylpenicilloic acid, sodium benzylpenicilloate (MDM) from Diater Laboratorios (Penicillin allergenic determinants (DAP) ® test). We followed the investigation protocol given by the manufacturer. Cutaneous tests were started with the major determinant. If the prick tests at different dilutions were negative, the testing was continued with intradermal and then patch tests. Each prick and intradermal test was read once 20 minutes elapsed since their application. Test results were also read at 24, 48, 72, and 96 hours for detecting delayed reactions. Patch tests were performed using the powdered culprit drug mixed into Vaseline (1:1). Allergens were applied to the upper back in individual round chambers (Curatest®, Spiromed Ltd.). Readings were performed at 48, 72, 96 hours, and 7 days. Although skin rashes occurring in mononucleosis are likely delayed-type reactions, we performed immediate readings, as clinical history can often be unreliable; patients are prone to mistaking intermediate reactions for delayed reactions.

Study II: Hyperuricemia and drug hypersensitivity

Lymphocyte Transformation Test

In our department, 335 patients with suspected allopurinol hypersensitivity were investigated by Lymphocyte Transformation Test (LTT) from January 2002 until January 2017. This *in vitro* method was also performed, 1-1.5 months after the cessation of skin eruptions, to determine T-cell proliferation as an indicator of drug sensitization, using the same method described previously with patients with infectious mononucleosis.

Drug allergy work up and clinical history

To determine the reliable diagnosis of allopurinol-induced adverse drug reactions, next to the evaluation of the above-described *in vitro* method, we applied drug allergy workup according to the advice of International Consensus on drug allergy consensus during the detailed data collection and analysis. By following the recommendations regarding drug hypersensitivity diagnosis, allopurinol could be identified as the culprit drug in 37 cases. To identify properly the Drug Hypersensitivity Syndrome cases among severe cutaneous adverse drug reactions, we applied the RegiSCAR criteria for DRESS. We were able to obtain a complete clinical history in all cases, including age, gender, evaluation of the clinical symptoms, pharmacologic history (allopurinol appliance, concomitant medications), and comorbidities, also results of routine blood and other examinations. To study the risk of adverse drug reaction induced by allopurinol in association with comorbidities, we have selected diseases from the van Walraven Elixhauser and Charlson-Romano Comorbidity Index. Categorical variables were evaluated with the Fischer's exact test (R Studio software). We calculated the odds ratio (OR) and p-values using the Fisher's exact test. Values which were regarded as statistically significant ($P < 0.05$) were noted in the study. Our treatment procedure was also recorded.

Histopathology

To the recognition of appropriate histologic signs of allopurinol-induced reactions, skin biopsies were performed in almost all cases. Punch or deep incisional biopsy samples were examined by histochemical staining and direct immunofluorescence (DIF) testing. The histopathology examination was done at our dermatopathology unit as part of a routine examination. Next to routine hematoxylin-eosin and periodic acid-Schiff/diastase staining, different special stains were also employed in the required cases, such as alcian blue, Congo red, Fontana-Masson, Gram, Perl's potassium ferrocyanide, phosphotungstic acid hematoxylin, and Verhoeff-van Gieson stain. CD1a, CD20, CD34, CD45, and CD68 helped us in immunohistopathology. Primary immunofluorescence assay was used to detect deposits of different immunoglobulin isotypes, such as immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) and complement proteins, as complement component 3 (C3c) in our skin biopsies.

HLA association

HLA-B*58:01 evaluations were carried out after recovery in eight patients, as well as in eight healthy study participants without allopurinol therapy. All subjects were of Caucasian origin. The detection of HLA-B*58:01 alleles was performed by DNA purification from a peripheral blood sample (QIAampR genomic DNA and RNA kits), then real-time polymerase chain reaction amplification and reverse hybridization using the PG5801 Detection Kit from the Pharmigene Inc.

4. Results

Study I: Infectious mononucleosis and drug hypersensitivity

10 patients who were treated at our clinic between 2002 and 2012 10 (mean age 22.9, range 15-35 years) with infectious mononucleosis and maculopapular rash were examined with drug allergy tests. All patients took antibiotics before the appearance of skin symptoms. The cutaneous eruptions developed a few days after the initiation of antibiotic therapy. In severe cases, confluence of the progressive maculopapular exanthems appeared on the trunk.

Histologic examination showed hydropic degeneration at the basement membrane and inflammatory infiltration around vessels with a few eosinophil cells. Histologic sections were stained with hematoxylin-eosin and studied with a brightfield microscope.

In all ten patients *in vitro* lymphocyte, transformation tests were performed with the suspected drugs. Amoxicillin/clavulanic acid enhanced drug-specific response in none of the cases. Increased lymphocyte proliferation was found in one peripheral blood sample after incubation with cefixime. Six out of the 10 patients with suspected sensitization to amoxicillin and negative LTT results were further investigated *in vivo* by prick, intracutaneous and patch testing. All six patients showed negative responses with prick tests. The intradermal tests resulted in positive reactions in four subjects. Patch tests were performed after negative prick and intracutaneous testing with negative results in the remaining two patients, verifying the development of sensitization to penicillin. We recognized positive skin reaction to MDM at 1:100 and 1:10 dilutions, and in one case with the undiluted form of MDM. In two cases, positive skin tests were detected to PPL at 1:100 and 1:10 dilutions, and at 1:10 dilutions. Patch tests were

performed after negative prick and intracutaneous testing with negative results in the remaining two patients.

It is important to notice that the *in vivo* investigations were carried out at least six months after the disappearance of the eruptions, which leads us to think that true drug sensitization developed instead of a transient loss of tolerance; a transient Th-1 lymphocyte-mediated delayed-type hypersensitivity reaction to the medication as discussed in the literature.

Study II: Hyperuricemia and drug hypersensitivity

Lymphocyte transformation test

335 patients, sent from different health care institutes with suspected allopurinol hypersensitivity, were investigated by Lymphocyte Transformation Test from January 2002 until January 2017 at our department. The number of cases was 89 between January 2002 and July 2009 and 246 between July 2009 and January 2017, reflecting the increased use of the drug in Hungary. A complete drug allergy work-up was done in 190 cases. Of the 190 patients, allopurinol could be identified properly as the culprit drug in 37 cases: 19 female and 18 male patients, the mean age was 70 years among female patients, ranging from 51 to 85 years, and the mean age was 67 years among male patients, ranging from 46 to 90 years. Lymphocyte Transformation Test with allopurinol was positive only in 4 cases out of 37. Of the 4 positive patients two had maculopapular exanthems, one patient was diagnosed with DRESS, and one with vasculitis.

Pharmacologic history

The starting, as well as the maintenance allopurinol dose, was high, 300 mg/day, among our patients. Only one patient with DRESS took 100 mg/day. According to our concomitant drugs' analysis, 8 drug categories were suspected of being relevant risk factors for developing allopurinol-induced hypersensitivity reactions: angiotensin-converting enzyme inhibitors, benzodiazepines, beta-blockers, diuretics, HMG CoA-reductase inhibitors (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase), non-steroidal anti-inflammatory drugs (NSAIDs), proton-pump inhibitors (PPIs), and thrombocyte aggregation inhibitors (antiplatelet drugs). Administration of benzodiazepines, diuretics, proton-pump inhibitors, thrombocyte aggregation inhibitors, and beta-blockers was significantly higher compared to the others.

Co-morbidity

Indication for allopurinol administration in the studied population was the prevention of the pro-inflammatory effect of hyperuricemia in all cases. Notably, only four of these patients had definitive gout disease (three from the generalized maculopapular exanthems group and one patient with SJS). The average serum urate level was 398.23 μmol/L among the patients. The following comorbidities could be risk factors for allopurinol-associated hypersensitivity reactions among our patients: heart diseases (congestive heart failure, cardiac arrhythmias, valvular disease), vascular disorders (cerebrovascular disease, coronary artery disease), hypertension, diabetes, liver disease, and renal failure. The presence of hypertension, renal failure, and vascular disorders was significantly high among the studied group.

Clinical characteristics

Cutaneous symptom onset occurred generally within the first 4 weeks of allopurinol therapy (average 3.6 weeks) in 28 patients, ranging from 1 to 8 weeks. We do not have exact information in 9 cases. Five patients, of whom two were diagnosed with erythroderma (ED), two with maculopapular exanthems (MPE), and one with Stevens-Johnson syndrome (SJS), were exposed to allopurinol repeatedly by mistake. On repeated exposure similar symptoms, but less severe, appeared to the initial ones, in 24 to 120 hours. The other patients remained symptomless after treatment and re-administration of all other drugs exception for allopurinol, indicating strongly that allopurinol was the causative agent. The distribution of clinical forms is described in Fig. 6: Severe cutaneous adverse drug reactions were found in 17 cases: 13 cases of DRESS, 1 case of AGEP, 2 cases of SJS and 1 patient had SJS/TEN overlap. The clinical features were generalized maculopapular exanthems among 16 patients and erythroderma among 2 patients (named exanthematous eruptions in the figure) and vasculitis in 2 cases.

Dermatopathology

The histological clues to allopurinol-induced hypersensitivity reactions were examined with the help of 29 skin biopsies (histochemical staining), accompanied by 26 biopsies for DIF testing. In all cases the histology showed interface dermatitis, characteristic histopathologic features in late type allopurinol induced reactions. Histologic patterns included apoptosis of basal keratinocytes, appearing as hydropic degeneration, individual and/or confluent necrosis of

keratinocytes, and ranging to full-thickness epidermal necrosis with intra- and/or subepidermal bulla formation. Papillary dermal edema and superficial intra- and perivascular lymphohistiocytic infiltrate with eosinophils and neutrophils were also present. In the case of AGEP, subcorneal and intraepidermal pustule formation and marked neutrophil counts were the additional characteristic signs. On direct immunofluorescence (DIF) examination mostly C3, in vasculitis IgM was also detected at the dermal-epidermal junction and in the walls of small dermal vessels.

HLA-B*58:01 allele

Eight of our patients with verified allopurinol sensitivity agreed to take part in our HLA-B*58:01 study. They had different adverse reactions and were from different genders: generalized maculopapular exanthems in 5 cases (two females and three male patients), drug hypersensitivity syndrome in 2 cases (one female and one male patient), and Stevens-Johnson syndrome in 1 case (female patient). The detection of HLA-B*58:01 alleles was positive only in a female patient who previously developed DRESS. Moreover, among the healthy donors in the control group, we also identified one subject with HLA-B*58:01 allele positivity.

Therapy

At first, we discontinued the administration of allopurinol together with the drugs that were suspected as possible cofactors. Our treatment procedure consisted of topical and systemic (oral or intravenous) corticosteroid therapy (methylprednisolone), with 0.4 mg/kg to 1.5 mg/kg starting daily dose according to the severity of the adverse reaction. Recovery of patients and reduction of steroid therapy lasted for weeks (on average 3 weeks), according to the symptoms. For the prevention of future reactions, patients were provided with information to strictly avoid allopurinol.

5. Discussion

Study I: Infectious mononucleosis and drug hypersensitivity

We aimed to find out whether true amoxicillin sensitization was developed for aminopenicillin among our patients. Evidence shows in the literature that the development of allergic reactions

to aminopenicillin during a florid viral infection is more prevalent than was believed previously. Although Renn et al. earlier demonstrated true sensitizations to amoxicillin in three patients with infectious mononucleosis and a clear history of amoxicillin intake with positive proliferative responses, we further investigated this phenomenon to provide more evidence. Our results add additional evidence that indeed in such patients, drug sensitization develops during the infection. The unquestionable proof of a drug allergy or hypersensitivity that has clinical relevance would require a challenge with the culprit drug, the clinical importance of skin testing and LTT to determine who will develop clinical symptoms is still not clear and only a large scale study in which patients would be challenged with the culprit drugs could answer this question. We cannot explain the negative results of LTT in all of these cases to penicillins. It may be that our method could be improved, but it may be that this group of patients is not the one in which LTT could work. We do get positive LTT results for penicillins, mostly in patients with immediate-type reactions. The two patients with negative *in vitro* and *in vivo* test results need to be further investigated by performing cutaneous tests with the culprit drug and if this was negative a drug provocation test should be applied to prove that neither amoxicillin side chain sensitization nor penicillin sensitization developed. In this work, our primary aim was to demonstrate that true sensitization can occur within mononucleosis infectiosa patients suffering from amoxicillin rash.

Study II: Hyperuricemia and drug hypersensitivity

Some studies found hyperuricemia to be an independent risk factor for coronary and heart diseases, heart failure, stroke, and cardiovascular death, which indicates the importance of preventive urate reduction. However, allopurinol is highly associated with cutaneous adverse drug reactions. Allopurinol can induce severe cutaneous adverse reactions, such as SJS, SJS/TEN overlap, TEN, DRESS, or AGEP. These are important life-threatening medical conditions with high mortality rates and poor prognoses. Since allopurinol prevention became generalized in Hungary, we found an increase in the allopurinol-hypersensitivity reactions in our region.

The elderly population was particularly susceptible to allopurinol hypersensitivity reactions (the mean age was 63 years among our patients), without any gender preference. Existing diseases seem to affect the development of allopurinol hypersensitivity, making this population

more vulnerable to severe drug reactions. The prevention of cardiovascular disorders in this population is also questionable. The presence of hypertension, renal failure, and vascular disorders (cerebrovascular disease, coronary artery disease) was significantly high in our studied group. Among the drugs that our patients were concomitantly taking, we found eight drug categories that could have affected on developing allopurinol-associated adverse reactions. Administration of benzodiazepines, diuretics, proton-pump inhibitors, thrombocyte aggregation inhibitors, and beta blockers was significantly frequent. These factors (concomitant disorders and their treatments) could influence renal clearance, thus the elimination of allopurinol. Higher starting and maintenance doses of allopurinol were also observed in our patients. Reduced renal clearance, competing molecules in the cytochrome p450 system, aldehyde oxidase, and renal transporters influence the metabolism and excretion of allopurinol leading to high allopurinol and oxypurinol plasma level. The risk of developing adverse reactions is elevated by the known toxic effect of the drug, primarily its oxypurinol metabolite. Oxypurinol-specific T-cell activation has been reported. Probably in young, healthy people allopurinol prevention therapy has much less risk and much more benefits. The "start low, go slow" principle in allopurinol therapy and early recognition of drug hypersensitivity symptoms are also important.

Prevention of non-predictable hypersensitivity can be challenging. A strong HLA-B*58:01 allele association was detected in Han Chinese, Thai, Japanese and Korean population, while a relevant but weaker association was found in patients of European ancestry. We found one HLA-B*58:01 allele positivity out of eight patients, who had DRESS, and one positivity among the eight healthy donors. While in the Asian population, SJS and TEN were the disorders associated with the HLA-B*58:01 allele, in the European population DRESS was also connected to this variant similar to our patient. Based on 16 individuals we cannot reach a reliable conclusion regarding the HLA-B*58:01 allele frequency in the Hungarian population. It is feasible, but probably not financially affordable to screen patients for the HLA-B*58:01 allele prior to starting allopurinol therapy. However, our data suggest that even with the detection of the HLA-B*58:01 allele, we cannot avoid the development of severe reactions, as they occur in patients without HLA-B*58:01 allele association.

In our cohort, clinical symptoms of allopurinol hypersensitivity were diverse. Aside from the delayed-type (type IV) adverse drug reactions, vasculitis was also found. The skin was always

involved in the hypersensitivity reactions and histologic examination confirmed the diagnosis, in addition to the clinical features and the history of drug intake. In all cases, the histology showed interface dermatitis, characteristic histopathologic features in late-type allopurinol-induced reactions. Histologic patterns included apoptosis of basal keratinocytes, appearing as hydropic degeneration, individual and/or confluent necrosis of keratinocytes, and ranging to full-thickness epidermal necrosis with intra- and/or subepidermal bulla formation. Papillary dermal edema and superficial intra- and perivascular lymphohistiocytic infiltrate with eosinophils and neutrophils were also present. In the case of AGEP, subcorneal and intraepidermal pustule formation and marked neutrophil counts were the additional characteristic signs. The severity of clinical features depended on the degree of basal keratinocyte necrosis. On direct immunofluorescence (DIF) examination mostly C3, in vasculitis IgM was also detected at the dermal-epidermal junction and in the walls of small dermal vessels.

Lymphocyte Transformation Test (LTT) was performed in all patients and the results indicated 53 % sensitivity and 96 % specificity for the LTT with allopurinol.

6. Conclusion

Our data demonstrate that *in vitro* testing, specifically lymphocyte transformation test is not sensitive enough in determining drug sensitization for penicillin in patients who develop skin symptoms during mononucleosis infection. *In vivo* tests should be performed to detect sensitization and indeed with skin tests, our results confirmed that sensitization to aminopenicillin may develop within infectious mononucleosis.

Adverse drug reactions are unpredictable, unwanted effects of medications, which can rapidly progress into life-threatening conditions. Due to the increasing number of allopurinol hypersensitivity, the cardiovascular preventive role of allopurinol in the elderly population who already developed cardiovascular diseases and other comorbidities requires more careful consideration. It is important to keep the “start low, go slow” therapy principle and monitor early symptoms of adverse reactions. HLA-B*58:01 allele screening is feasible, but probably not financially affordable, as it cannot be relied upon to prevent the development of severe reactions.

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