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

Ph.D. Thesis

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List of publications

This doctoral thesis is based on the following publications

I. **Ónodi-Nagy K.**, Kinyó A., Meszes A., Garaczi E., Kemény L., Bata-Csörgő Zs. Amoxicillin rash in patients with infectious mononucleosis: evidence of true drug sensitization. *Allergy, Asthma and Clinical Immunology.* 2015; 11 (1). **IF: 2.283**

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).

III. **Ónodi K.**, Bata-Csörgő Zs. Gyógyszerallergia: nemzetközi konszenzus. [Drug allergy: international consensus]. *Dermatology and Venereology Journal HU.* 2014; 90(4), 133–137.

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II. **Ónodi Katinka**: A gyógyszerallergiák *in vitro* diagnosztikája. [Drug allergy: *in vitro* diagnostic procedure]. *Diploma work at the Department of Dermatology and Allergology, Szeged.* 2012. Supervisor: Dr. Bata-Csörgő Zsuzsanna

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Tartalomjegyzék

List of abbreviations

ACE-I: Angiotensin-converting enzyme inhibitor

AGEP: Acute generalized exanthematous pustulosis

DEJ: Dermal-epidermal junction

DHS: Drug hypersensitivity syndrome

DIF: Direct immunofluorescence

DRESS: Drug reaction with eosinophilia and systemic symptoms

EBV: Epstein-Barr virus

ED: Erythroderma

EM-like: Erythema multiforme-like

F: Female

FUR: Furosemide

GM-CSF: Granulocyte-macrophage colony-stimulating factor

HCT: Hydrochlorothiazide

HLA: Human leukocyte antigen

HMG CoA R-I: 3-hydroxy-3-methyl-glutaryl -coenzyme A reductase inhibitor

IND: Indapamide

LTT: Lymphocyte transformation test

M: Male

MDM: Minor determinant mix

MPE: Maculopapular exanthems

N/A: Not applicable

NSAID: Non-steroidal anti-inflammatory drug

PPIs: Proton-pump inhibitors

PPL: Poly-L-lysine

SCORTEN: Severity-of-illness score for toxic epidermal necrolysis

SJS: Stevens-Johnson syndrome

SPL: Spironolactone

SPF: Superficial

TEN: Toxic epidermal necrolysis

TNF: Tumor necrosis factor

1. Introduction

1.1. Classification of drug hypersensitivity reactions

Drug hypersensitivity reactions are based on unwanted stimulation of immune or inflammatory cells after exposure to a medication. Their 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, T cell receptors (pharmacological interaction with immune receptors concept), or interactions with enzymes or receptors of inflammatory cells can also lead to drug hypersensitivity reactions [1], [2].

The mechanisms underlying the development of a hypersensitivity reaction are complex, so the clinical picture shows heterogeneity. Different attempts have been taken to sub-classify drug hypersensitivity reactions for an earlier diagnosis, adequate management, and possible prevention.

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, inflammatory cells, and on immunologic mechanism [1]–[4].

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 incriminated drug. Based on the immunologic mechanism, reactions have been divided into four main categories, named I to IV hypersensitivity reactions. Most drugs cause mainly just one type, although certain medications can induce all four types. The immune reactions can be mixed, the character of a hypersensitivity reaction depends on the dominant immune mechanism (Table 1) [1]–[4].

Reaction type	Immune response and pathophysiology	Clinical manifestation
Type I	IgE-mediated reaction – mast cell and basophil degranulation	urticaria, angioedema, anaphylaxis, bronchospasm
Type II	IgG and complement-dependent cytotoxicity FcR, phagocytes, natural killer cells	cytopenia
Type III	IgM or IgG and complement FcR-mediated reaction Deposition of immune complexes	vasculitis, serum sickness, hypersensitivity pneumonitis, drug-induced lupus, urticaria, Arthus reaction
Type IV a	Th1 cells – monocytic inflammation (macrophages) IFN-γ and TNFα	allergic contact dermatitis
Type IV b	Th2 cells – eosinophilic inflammation IL-4, IL-5, IL-13	maculopapular exanthema, DRESS
Type IV c	Cytotoxic T cells – keratinocyte death mediated by $CD4^+$ /$CD8^+$ CTL perforin, granzyme B, FasL	maculopapular exanthema, pustular exanthema, SJS, TEN
Type IV d	T cells – neutrophilic inflammation IL-8/CXCL8, IL-17, GM-CSF	AGEP, Behcet disease

Table 1: Classification of drug hypersensitivity reactions

Figure adapted from the position paper International Consensus on drug allergy [5]

TYPE I HYPERSENSITIVITY

Type I reactions require the presence of drug-specific IgE. Once formed, drug-specific IgE occupies surface receptors on mast cells and basophils. Re-exposure to the drug causes crosslinking of IgE bound to high-affinity receptors on the surface of sensitized mast cells and basophils leading to the release of preformed vasoactive mediators such as histamine, tryptase, and chymase. IgE-mediated reactions therefore generally require prior exposure to the drug, although sensitization may have occurred from exposure to a cross-reactive compound. The released mediators cause vasodilatation and increased vascular permeability. These lead to the development of urticaria, angioedema, bronchospasm, hypotension, and/or gastrointestinal symptoms within 1 to 6 hours after the last intake of the drug. Anaphylaxis is the most severe presentation of type I hypersensitivity reactions. These are immediate reactions, and the time

to onset depends on the route of administration, intravenously in seconds, orally in minutes to hours. The initial reaction is followed 4 to 8 hours later by a late-phase reaction, because of cytokine releases, such as IL-1, IL-4, IL-5, GM-CSF, and TNF-alpha. Beta-lactam (penicillin, cephalosporins) and quinolone antibiotics, neuromuscular blocking agents, foreign proteins (cetuximab, rituximab), and platinum-containing chemotherapeutic agents (carboplatin, oxaliplatin) are commonly implicated in type I hypersensitivity [1], [3]–[5].

TYPE II HYPERSENSITIVITY

Type II reactions are uncommon, delayed cytotoxic reactions in which host cells are destroyed through complement-mediated reactions, antibody-dependent cell-mediated cytotoxicity, or antibody-mediated cellular dysfunction. Reactions arise when drugs bind to surfaces of certain cells and act as antigens. Host cells coated with antigens bind to IgG or rarely IgM antibodies, leading to classic complement pathway activation, resulting in subsequent lysis of the host cell via membrane attack complex (C5-C9).

The clinical features vary widely in severity from asymptomatic to fulminant illness. These reactions result in anemia, thrombocytopenia, and neutropenia since these are the most often affected cell types. Specific clinical manifestations depend on the cell type involved. Drugs implicated in drug-induced immune hemolytic anemia are beta-lactam antibiotics, sulfonamides, vancomycin, heparin, abciximab, NSAIDs, carbamazepine, gold compounds, quinine, and quinidine [1], [4], [5].

TYPE III HYPERSENSITIVITY

Reactions are mediated by antigen-antibody complexes (IgG or IgM immune complexes) which activate the complement system and precipitate in various tissues (blood vessels, joints, renal glomeruli). These small immune complexes bind to Fc-IgG/IgM receptors on inflammatory cells and/or activate complement leading to inflammation and tissue injury by active neutrophils.

These reactions usually occur after high-dose, prolonged drug administration. Signs and symptoms take one to few weeks to develop after drug exposure, 7-8 days for serum sickness or urticaria vasculitis, and 7-21 days after the start of the eliciting drug for vasculitis, as a

significant level of antibody is needed to generate symptoms. Re-exposure to the same drug can cause more rapid and severe recurrence.

Drug-induced vasculitis presents as palpable purpura, petechial, and/or urticaria in the skin, with fever, arthralgias, lymphadenopathy, elevated erythrocyte sedimentation, rate and low complement levels. Pruritic lesions affect the lower extremities. Inner organ involvement is rare, if yes, the gastrointestinal tract and kidneys are involved. Common culprit medications are penicillin, cephalosporins and sulfonamides, loop and thiazide diuretics, phenytoin, and allopurinol [1], [4], [5].

TYPE IV HYPERSENSITIVITY

Type IV hypersensitivity reactions involve the activation and expansion of T cells, which require hours or days, therefore they are named delayed-type hypersensitivity reactions. In some cases, macrophages, eosinophils, or neutrophils are also involved. Their severity ranges from mild to life-threatening. Severe cutaneous adverse drug reactions are associated with high morbidity, mortality, healthcare costs, and challenging management. This group covers a broad spectrum of entities, consisting of drug hypersensitivity syndrome (DHS), acute generalized exanthematous pustulosis (AGEP), Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN). The time to symptom onset, from 48-72 hours to days or weeks, depends in part on the number of drug-activated T-cells. On re-exposure, clinical signs may appear within 24 hours. These are polyclonal responses and clinical features occur rapidly if the drug stimulates a large number of different T-cell clones. Medication that activates a few clones, only causes clinical symptoms until these T cells have proliferated for several weeks. In fulminant immune reaction, the clinical presentation results from uncontrolled expansion of oligoclonal T cells that have been massively stimulated by the drug, reminiscent of super antigen-like stimulation. As the skin is a depository for a large number of cells, dermatologic involvement is common. Many cutaneous T cells are primed memory-effector cells, which react rapidly with immunogenic agents that penetrate the skin barrier or appear in the skin from its circulation.

A subdivision of type IV hypersensitivity reactions has categories distinguished by the effector cells and mediators involved, together with the resulting clinical presentation. These categories are named from a to d. Type IV/a reaction is mediated by Th-1 cell activation, leading to

monocytic inflammation with IFN-gamma as the dominant cytokine. Contact dermatitis, eczema is a common clinical form of this type. Type IV/b reaction is induced by Th-2 cell activation and it is mediated by eosinophils with IL-4 and IL-5 involvement. Maculopapular rash and drug hypersensitivity syndrome develop due to this reaction. In Type IV/c reaction cytotoxic T cells rule the picture with perforin and granzyme B as relevant effector molecules, leading to keratinocyte death due to CD4 or CD8 cells. This can appear in a maculopapular rash or pustular exanthema, but the most severe clinical outcome is SJS and TEN. In Type IV/d T cell-mediated reaction neutrophil cell and IL-8/CXCL8 involvement are relevant in developing AGEP [1], [3]–[6].

Maculopapular rash

Signs and symptoms occur one to several days after exposure in MPE, and two to six weeks in DRESS. They appear in 1 or 2 days in already sensitized individuals.

Maculopapular exanthema, also called morbilliform drug eruption, is the most frequently seen pattern of drug-induced skin eruptions and arises from type IV immunologic reactions, as well as from other mechanisms. The term maculopapular rash includes exanthems with varying degrees of cell infiltrations and thus papular components. Clinical presentation varies from an erythematous rash (mimicking viral or bacterial exanthem) to generalized symmetric eruptions, and confluent erythematous plaques. Pink to red macules and papules start to appear usually on the trunk and then spread to extremities, neck, and head. Purpuras may appear on the lower extremities. Symptoms tend to progress, even if the drug has been withdrawn, over a few days before regressing over a two weeks often accompanied by desquamation. Skin signs may be associated with mild fever and itching. Commonly implicated drugs are aminopenicillins, cephalosporins, sulfonamides, carbamazepine, allopurinol, and NSAIDs [1]–[3].

Drug hypersensitivity syndrome or drug reaction with eosinophilia and systemic symptoms

Drug reaction with eosinophilia and systemic symptoms (DRESS), also known as drug hypersensitivity syndrome (DHS) is a dangerous delayed drug hypersensitivity reaction. Often appears after a long latency period, two to six weeks of uncomplicated drug treatment, at which point some individuals suddenly develop signs and symptoms of a fulminant immune reaction.

Severe cutaneous adverse reactions due to drugs, despite their low annual incidence, can be life-threatening and responsible for severe, potentially chronic sequelae. The incidence of DHS reaction elicited by antiepileptics is about one per 1000 to one per 10000 people. DHS has a heterogenous presentation with visceral involvement and biological abnormalities, with or without dermatological signs. Skin eruptions can be preceded up to two weeks by a prodromal phase, including fever, lymphadenopathy, influenza-like symptoms, burning sensation, and/or pruritus. Dermatological signs consist of facial and distal edema, erythroderma, purpura, pustules, and focal mucosal involvement may occur.

Syndrome-specific internal organ involvement results from eosinophil or lymphocyte tissue infiltration. Liver involvement is frequently observed, mainly hepatic cytolysis, cholestasis, or rarely fulminant hepatic failure. Interstitial nephritis represents kidney involvement. Lungs are affected in up to 15% of the cases, lung involvement is characterized by dyspnea, cough, eosinophilic pneumonitis, and respiratory failure is rare. Cardiac enzyme abnormalities, myocarditis, and pericarditis can be fatal. Rare visceral effects, which can be neurological, muscular, hemophagocytic, or pancreatic are associated with poor prognoses. The presence of atypical, activated CD8+ lymphocytes is a more consistent finding, than peripheral eosinophilia, which may persist for months after drug withdrawal.

To reduce misdiagnosis, different diagnostic criteria have been proposed previously. In 2007, the RegiSCAR group, a multinational effort that collects data on cases of severe cutaneous adverse drug reactions, proposed a set of diagnostic criteria and a scoring systems to help classify as definite or probable DRESS or exclude the diagnosis [7]. Drugs that have been implicated in causing DHS are several antiepileptics (carbamazepine, phenytoin, lamotrigine, phenobarbital), minocycline, dapsone, abacavir, nevirapine, and allopurinol [3]–[6].

Stevens-Johnson syndrome and toxic epidermal necrolysis

SJS and TEN are severe, potentially life-threatening blistering dermatitides that occur 4 to 28 days after the start of the eliciting drug. They are characterized by fever and mucocutaneous lesions leading to necrosis and sloughing of the epidermis. Clinical classification is defined by the extent of body surface area with skin detachment. SJS involves less than 10 % of the body surface area with skin detachment, whereas TEN involves more than 30 % of the body surface area with skin detachment. SJS/TEN overlap represents cases whereby there is between 10% and 30% of body surface area involved. Previously considered to be on the continuum of the

same disorder, erythema multiforme is now thought to be a distinct entity. In about 30% of the cases, no causative medication is identified and in 15% of the cases, drug responsibility is deemed unlikely (e.g. mycoplasma pneumoniae infection in children).

In the initial phase patients present with an influenza-like illness which may include fever and burning sensation, skin pain. This prodrome precedes the development of cutaneous findings by 1 to 3 days. Initially in SJS/TEN, lesions are first present on the face, upper trunk, and proximal part of extremities before spreading to other areas. The distribution is symmetric and usually spares the scalp, palms, and soles. Initial lesions are erythematous, irregularly shaped, dusky-red macules, or target-like lesions with dark centers. Over time, sometimes within hours, vesicles and bullae form, necrotic lesion confluence and leads to extensive erythema, flaccid blisters, and large epidermal sheets, revealing areas of red dermis. Epidermis sloughs off under lateral pressure, which is called positive Nikolsky's sign. The Asboe-Hansen sign may also be present, in which lateral pressure on the edge of the blister will make the blister spread into uninvolved skin.

In 80% to 90% of the cases, patients will have one or more mucosal involvement, often preceding skin eruptions. Erythema, vesicles, bullae, and erosions involve ocular, pharyngeal, genital, and anal mucous tissue, associated with pain and dysfunction. The eyes may demonstrate conjunctival lesions, such as hyperemia, erosions, conjunctival and periorbital edema, crusting, development of pseudo membrane, photophobia, and tearing also occurs. Lips can develop a vermillion border and greyish pseudo membrane coat next to the inner oral hemorrhagic erosions with crusting. Visceral involvement consists of transient liver or renal enzyme increase, and bronchial and epithelial necrosis. Specific acute visceral failure is rare but should be remembered after eliminating bacterial or viral superinfection.

The severity-of-illness score for toxic epidermal necrolysis, SCORTEN was developed to determine the severity and predict prognosis, with the use of seven independent variables (age, associated malignancy, heart rate, serum bicarbonate, glucose, and blood urea nitrogen, detached or compromised body surface). With increasing scores, the mortality rate increases, over 5 is greater than 90% mortality [1], [4]–[6], [8].

Acute generalized exanthematous pustulosis

Acute generalized exanthematous pustulosis (AGEP) develops due to an IV/d type of immune reaction caused by exposure to a drug with a very short latency period, frequently, but not exclusively, 1 to 2 (with a maximum of 11) days after the start of the eliciting medication. It is a rare type of severe cutaneous adverse drug reaction characterized by high fever and numerous small, superficial, primarily non-follicular sterile pustules, arising on large areas of edematous erythema. The rash first involves major intertriginous zones, in the axillary, submammary, and inguinal regions, then trunk or upper extremities. In the early stage, superficial epidermal detachment can appear due to pustule confluence, whereas only post-pustular desquamation is observed at the late stage. In about one-fourth of patients, pustules or erosions were seen on mucous membranes, usually orally. Evidence of systemic inflammation includes the development of fever, leukocytosis with elevated neutrophils (also eosinophils in more than a third of cases), and C-reactive protein. Relapse of pustular eruption without drug re-challenge is the most reliable sign to reject a diagnosis of AGEP. Lesions typically spontaneously regress in 2 weeks after discontinuation of the eliciting drug with the development of collarette desquamation. The mortality rate of the disease is about 5% and death usually occurs in patients with several comorbidities. It is considered to be less severe than SJS, TEN, and DRESS. Calcium channel blockers, antimalarials, and amoxicillin are the most frequently reported triggers of AGEP [1], [4]–[6].

Timing of symptom onset

A new classification of drug hypersensitivity is based on phenotypes, focuses on symptoms and the time between drug treatment and symptom onset, and endotypes, based on cellular and biological mediators, biomarkers as well as a genetic predisposition to elucidate underlying molecular pathways and to evaluate the risk of developing drug hypersensitivity in case of drug re-exposure. Combining classic and modern approaches to drug allergy is necessary for its better understanding and management [1], [4], [9].

IMMEDIATE REACTIONS

Reactions that occur during or within one to six hours after drug administration are classified as immediate reactions. Immediate phenotype is typically a result of IgE-mediated mast cell

activation (epitope-specific IgE). The period of one hour identifies the majority of IgE-mediated reactions, which carry the risk of anaphylaxis in case of re-exposure. Histamine release is observed as a result of non-IgE mediated direct mast cell/basophil activation, induced by direct complement activation or reactions due to drugs with tetrahydroisoquinoline motifs which signal through the human G-protein-coupled receptor. The immediate phenotype includes a third group, the non-immunologically mediated (cross-reactive) hypersensitivity reactions to non-steroid anti-inflammatory drugs (NSAIDs), NSAIDs exacerbated respiratory disease, NSAIDs exacerbated cutaneous disease, and NSAIDs induced urticaria/angioedema. They share similar anti-inflammatory mechanisms related to the inhibition of cyclooxygenase-1, an enzyme which is responsible for the generation of prostaglandins and thromboxane.

IgE-mediated reactions generally require previous exposure to the drug, however, cross-reactivity has been described between drugs and other allergens. Clinical features are attributable to the vasoactive mediators. The common signs are cutaneous symptoms, like urticarial rash, pruritus, flushing, angioedema of laryngeal tissues, face and extremities, respiratory symptoms, like wheezing, edema leading to throat tightness with stridor or asphyxiation, hypotension, and gastrointestinal symptoms. Due to the severity of the symptoms reactions are graded from one to level three, mild/ grade I includes skin symptoms or only one affected organ system, moderate/ grade II, when two or more organ systems are involved without vital sign changes, and severe / grade III when more organ systems are involved with life-threatening vital sign changes.

A group of symptoms, such as fever, chills, and non-determined pain have been reported to monoclonals, oxaliplatin, and taxanes, this is known as cytokine storm-like reactions, resulting from the release of proinflammatory cytokines which activate macrophages, and immune cells via Fcγ receptor [1], [9], [10].

DELAYED REACTIONS

Reactions appearing after 6 hours are classified as delayed, although they typically develop several days or weeks after cessation of drug treatment. On re-challenge, symptoms may appear within 24 hours. Type IV reactions are classically known as delayed type reactions. In contrast to type I, II, and III hypersensitivities they are not mediated by antibodies and have a more heterogeneous presentation, type IV drug reactions involve the activation and expansion of T

cells, which requires time. In some cases, macrophages, eosinophils, or neutrophils and toxic metabolites are also involved. Type IV reactions have many different clinical forms, which vary in significance from inconvenient to life-threatening.

This classification divides them into two main groups on behalf of the involved organs. Single-organ involvement forms include maculopapular eruptions, contact dermatitis, delayed urticaria, baboon syndrome, drug fever, isolated drug-induced hepatitis, isolated interstitial nephritis, and isolated pneumonitis. In the multiple organ involvement group (systemic delayed reactions), beyond the cutaneous signs, liver, lungs, and kidneys involvement or hematological alterations are also presented. Among them, pharmacogenomic (HLA associations) and viral titer screening is necessary among severe cutaneous adverse reactions, like DRESS, AGEP, and SJS/TEN.

Type II and III immunologic reactions, described above in the immunologic mechanism section, are all considered delayed reactions [1], [6], [9].

1.2. Diagnostic procedure

Different reaction types show different clinical manifestations and timings; therefore, the underlying mechanisms should be taken into consideration during the allergological work-up. The diagnosis is based on clinical history, clinical manifestation, and if possible on *in vivo* and *in vitro* biological tests. When properly performed in specialized centers, a reliable diagnosis is often possible and safe alternative medication can be administered [5], [11].

Clinical history and manifestations

A detailed clinical history is the most important step toward an accurate diagnosis of drug hypersensitivity reactions. Clinicians in all disciplines of medicine learn the importance of medical history and anamnesis of a patient. In addition to the obligatory and standard patient information assessment, in case of possible drug hypersensitivity assessment must include a series of relevant and specific drug-related questions. The European Network on Drug Allergy Questionnaire helps to harmonize the drug hypersensitivity diagnostic procedure [5].

It must be carefully obtained and should include symptomatology: symptom chronology (previous exposure, time between the last dose and the onset of symptoms), all drugs taken

(both at the time of reaction onset and other medication of the same class taken since), previous allergy associated or not with medications, disorders which can be aggravated by the intake of certain drugs (like NSAID intake in chronic urticaria/ rhinosinusitis), photographs are helpful if the patient is not seen during the symptomatic phase.

The clinical picture of these reactions is very heterogeneous, mirroring many distinct pathophysiological events. Recognition of clinical and biological danger signs suggesting severe cutaneous and/or systemic adverse drug reactions is crucial.

When a patient is seen during the hypersensitivity reaction, the suspected drugs should be stopped while considering the benefit/risk balance analysis into consideration. Clinical history can be unreliable because different drugs are frequently administered simultaneously and any of them can account for the symptoms, although often with very different a priori probabilities. History can also be imprecise in many cases.

An accurate diagnosis of drug hypersensitivity reactions allows the implementation of the best measures required for prevention and treatment. Simply avoiding the drug is not always an option [3], [5], [11].

In vitro testing

Given the limited sensitivity and possibility of skin testing, the potential unreliability of clinical history, and the danger or contraindication of drug provocation tests, it would be highly advantageous to have discriminating biological tests available to determine the culprit drug. The currently available *in vitro* methods for drug hypersensitivity testing lack sensitivity, although they are considered to be quite specific. There is no definite method to predict the immunogenic potential of a drug. There is no consensus regarding under which circumstances these tests are reliable. A negative result does not exclude drug allergy, while a positive result shows sensitivity to the medication, but does not reliably confirm its causality.

In vitro methods for evaluating drug hypersensitivity reactions depend on the underlying mechanisms, the participating cells, and the chemically varied range of biologically active agents that may be released during adverse reactions.

Mast cell and basophil cell activation have a major role during an immediate, IgE antibody-mediated adverse reaction. Detection of drug-related IgE antibodies (radioimmunoassay, fluorescent enzyme, fluorescent enzyme immunoassay). and from a large, assorted group of

chemically varied liberated mediators, detection of tryptase enzymes (fluorometric enzyme immunoassay), histamine (radioimmunoassay, enzyme-linked immunosorbent assays), and leukotrienes (cellular allergy stimulation test and enzyme-linked immunosorbent assays) could be valuable diagnostic aids. The basophil activation test became a reliable diagnostic tool for immediate hypersensitivity. The test is based on the determination of basophil cell activation or degranulation markers by flow cytometry after drug stimulation.

In non-immediate hypersensitivity reactions, most of the *in vitro* tests are based on reproducing T cell activation *in vitro* and measuring inflammatory and cytotoxic mediator release by different assays. For delayed, T-cell mediated, reactions lymphocyte transformation test (LTT) and nonproliferation-based tests for detecting released mediators and cell activity by flow cytometry, enzyme-linked immunosorbent spot assay, and immunosorbent assay are typically used. As several strong genetic associations with HLA alleles have been discovered in susceptibility to different forms of drug hypersensitivity reactions, searching for genetic markers may prove helpful in diagnostics.

Although type II and type III drug hypersensitivity reactions are not as common as others, they still exist. Coombs' test, *in vitro* hemolysis test, determination of complement factors, and circulating immune complexes can be performed in specialized centers to diagnose these reactions. Detection of drug-specific IgG and IgM antibodies is of interest in cases of drug-induced cytopenia, type III hypersensitivity reactions to vaccines, or allergies to dextran, although they are lacking sensitivity and are not widely available [3], [5], [11].

Lymphocyte Transformation Test

Lymphocyte Transformation Test (LTT) is often considered to be the only reliable 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%, positive predictive value is 92.3%, while its negative predictive value is 63.2%. Higher values were found among mild and moderate delayed reactions. The test is based on the detection of lymphocyte proliferation upon stimulation with the specific drug. The detailed methodology will be described below, in the materials and methods section of our study [3], [11].

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 [12]. 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 antibiotics, metamizole sodium monohydrate, acetylsalicylic acid, and diclofenac were equally high in both periods (Figure 1).

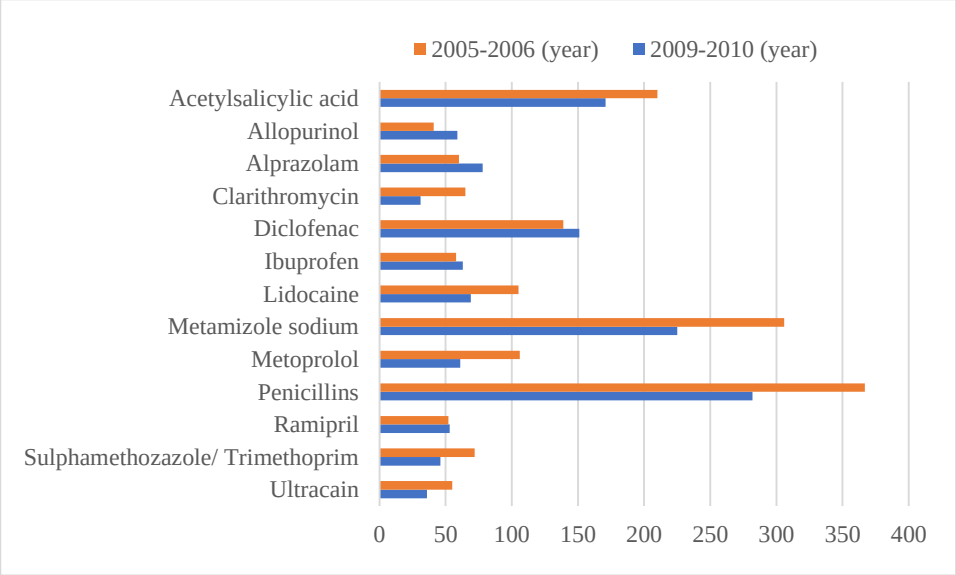


Figure 1: The number of LTT tests with the most frequently suspected drugs in two different yearly periods [12]

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. The following drugs were investigated: metamizole sodium monohydrate, diclofenac, acetylsalicylic acid, penicillin antibiotics, allopurinol, ibuprofen, metoprolol, ramipril, perindopril, alprazolam and lidocaine (Table 2). 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 [12].

	Metamizole sodium monohydrate	Diclofenac	Acetylsalicylic acid	Penicillin	Allopurinol	Ibuprofen	Metoprolol	Ramipril	Alprazolam	Lidocaine	**Number of reactions**
Positive and Negative LTT results	**59**	**43**	**41**	**36**	**27**	**21**	**21**	**16**	**12**	**10**	**286**
Positive LTT results	**13**	**4**	**6**	**2**	**2**	**1**	**2**	**0**	**1**	**0**	**31**
Maculopapular exanthema	**3**	**1**	**3**	**2**	**2**	**1**	**1**	0	0	0	**13**
Urticaria	**3**	**1**	**1**	0	0	0	**1**	0	0	0	**4**
Angioedema	**1**	0	**1**	0	0	0	0	0	0	0	**2**
Urticaria and angioedema	**2**	**1**	**1**	0	0	0	0	0	**1**	0	**5**
Fixed drug eruption	**1**	0	0	0	0	0	0	0	0	0	**1**
Vasculitis	**2**	0	0	0	0	0	0	0	0	0	**2**
Granulomatous reaction	**1**	0	0	0	0	0	0	0	0	0	**1**
Anaphylaxis	0	**1**	0	0	0	0	0	0	0	0	**1**

Table 2: Drugs, LTT, and clinical symptoms [12]

The 10 most frequent culprit drugs and the caused drug hypersensitivity reactions in 2010 at our department correlated to the LTT results.

In vivo testing

SKIN TESTS

After gathering and recording the detailed clinical history, the usual next step in the diagnostic procedure is to decide whether the available information suggests a hypersensitivity reaction. Testing subjects without a prior history of an allergic response to a drug is not supported by any available study or societies. Given the recent improvement of *in vitro* tests for evaluating drug hypersensitivity reactions, we may take into consideration, whether to start with *in vitro* testing (Figure 2). Skin tests have to be applied depending on the suspected pathomechanism of drug hypersensitivity reaction. They should be performed 4-6 weeks after the reaction. Antihistamines, corticosteroids, beta-adrenergic blocking agents, and with no universal agreement, tricyclic antidepressants must be discontinued before skin testing. The optimal drug test concentration, the highest concentration which produces no skin reaction in healthy individuals, but elicits a positive response in patients allergic to the drug, must be determined. Patient's vital parameters should be measured and recorded before, during, and after testing.

The risk of systemic reactions is lower in skin prick and patch testing than with intradermal testing. Pregnancy, young age, history of previous anaphylactic reactions, or uncontrolled asthma should be considered at higher risk of systemic and anaphylactic reactions [3], [5], [11].

Skin prick test

Skin prick and intradermal testing are particularly useful for reactive haptens to demonstrate an IgE-dependent hypersensitivity. Therefore, in immediate drug hypersensitivity reactions, a prick test is recommended for initial investigation due to its simplicity, rapidity, low cost, and high specificity. The test should be performed on the volar region of the forearm, or the back. Next to the prick test solution or solutions of the drug or drugs, positive and negative controls are used. Histamine (10 mg/ml) serves as a positive control, and physiological saline or glycerin-saline is used as a negative control. Solutions are placed on the skin and a sterile lancet with a 1 mm diameter is passed through the drop pricking the upper layer of the skin without drawing blood. The remaining solution on the skin is wiped away after 10 minutes. Skin prick tests should be read once 15-20 minutes elapsed since their application. A late reading is recommended at 24, 48, 72, and 96 hours for detecting delayed reactions. Late-reading is often necessary with antibiotics. Usually, erythema or wheal appears, but in case of delayed reactions

induration, papulation, and vesicles may also present. In general, if a wheal diameter is at least half of the diameter resulting from the positive control or a wheal diameter is at least 3 mm greater than the negative control, the test result is considered positive [3], [5], [11].

Intradermal testing

Intradermal tests are undertaken when skin prick tests elicit no reaction. They provide enhanced sensitivity for drug-specific IgE compared to prick tests. Intradermal tests should be performed with the intravenously injectable form of the medication whenever possible. The sensitivity and predictive value vary, depending on the clinical manifestation and on the drug itself. Its positive predictive value is high in general, but negative results cannot necessarily rule out hypersensitivity. Skin prick and intradermal tests appear to be a reliable option for beta-lactam antibiotics, neuromuscular blocking agents, platin salts, perioperative drugs, and heparins. There is no general agreement on skin testing in suspected radiocontrast media allergy, some consider it as nonallergic, anaphylactoid. In delayed-type reactions, T-cell-dependent mechanism manifesting especially with maculopapular exanthema, late-reading intradermal tests should be performed (next to patch testing). Testing is normally contraindicated in patients with prior history of SJS, TEN, or vasculitis, in necessary cases, special judgment and safety precautions are needed. Tests should be performed after a 20 minutes break from the prick test's immediate reading. Solutions should be sterile and prepared no longer than 2 hours before administration. Solutions (0.02-0.05 ml) are injected intradermally to the skin surface of the forearm or back, forming a small blister or bleb, by using a 25-gauge needle (15-20^{o} angle). If histamine is used intradermally as a positive control, 0.01-0.1 mg/ml base should be applied. We start with dilute solutions, generally with a dilution of 1:100 of the prick test solution, then increase the concentration in a logarithmic way until reaching the final concentration. If a positive reaction is seen throughout the process, testing should be stopped. The test should be read similarly to the prick test reading. The appearance of erythema or wheal with a diameter at least twice that of the initial bleb is considered a positive reaction [3], [5].

Patch test

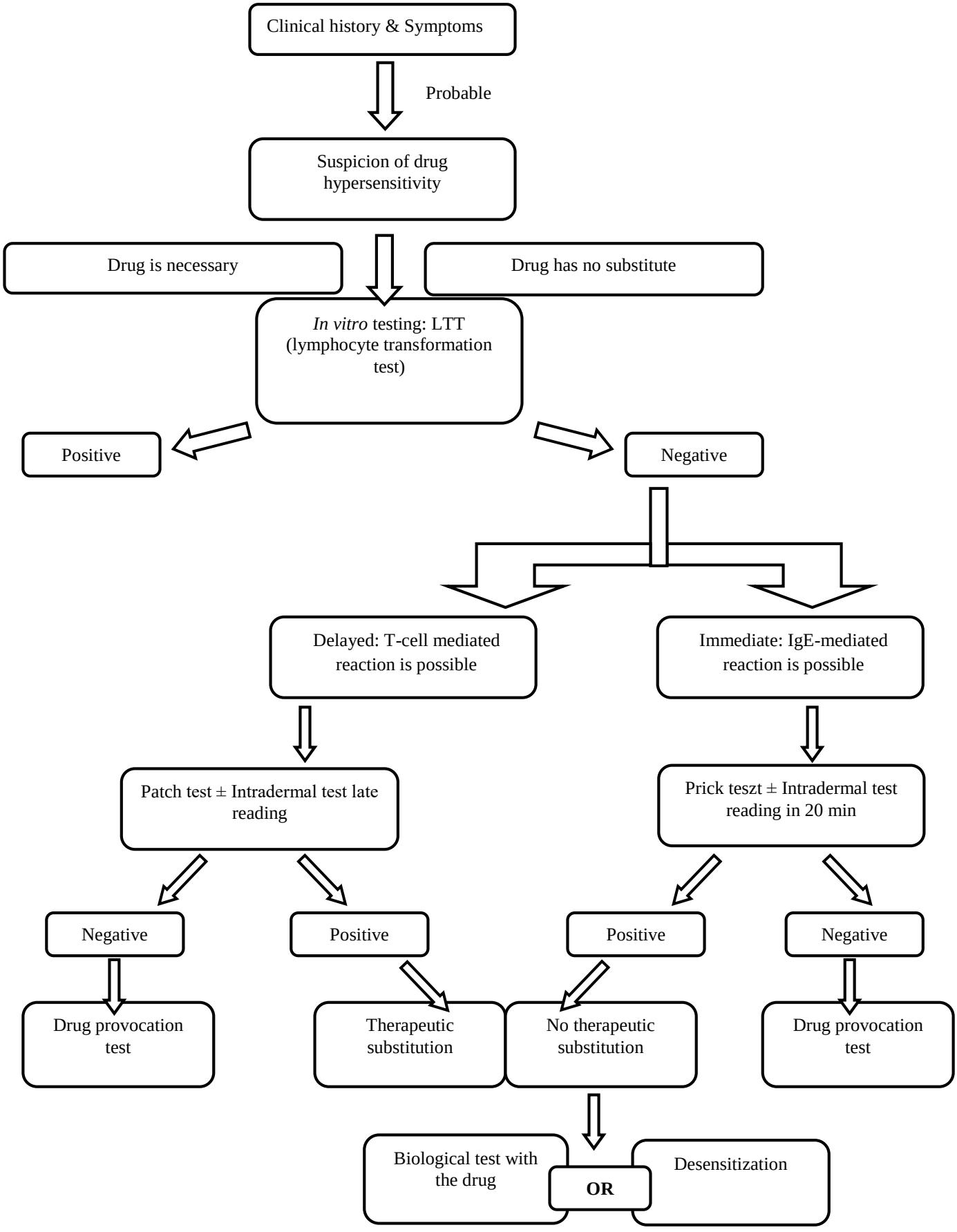
As adverse drug reactions affect the skin frequently, patch testing is both a screening test for hypersensitivity and a provocation test in the target organ, the skin. The general specificity and

sensitivity range from 70% to 80%. This depends on the prior clinical manifestation, the drug itself and its concentration, the vehicle used, and the skin test site. The test is valuable for investigating delayed and mainly T-cell mediated reactions, such as allergic contact dermatitis, maculopapular rash, AGEP, DRESS, fixed drug eruption, and photosensitivity. It is thought to be less useful in investigating cases of SJS, TEN, erythrodermic, or urticarial reactions. Patch test should be performed 6 weeks to 6 months after cessation of any cutaneous drug reaction, 4 weeks after discontinuing immunomodulating medications (e.g.: corticosteroids, cyclosporine), and 3 days after discontinuing antihistamine therapy [3], [5], [13].

DRUG PROVOCATION TEST

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, skin tests are not possible or their sensitivity may be suboptimal, a drug challenge test may be performed to eliminate any suspicion of allergic drug sensitivity. Drug challenge is also required when a drug's metabolite is responsible for the symptoms or when genetic disposition has a major role in hypersensitivity development. The test is considered to be the gold standard in the definitive diagnosis of drug hypersensitivity reactions. Drug challenge is contraindicated in non-controllable and severe life-threatening reactions, such as SJS, TEN, DRESS, AGEP, exfoliative dermatitis, vasculitis, any internal organ involvement, hematological reactions or during pregnancy. Patients should be in good health before testing. The route of administration depends on the drug, challenge can be oral, subcutaneous, intramuscular, intravenous, bronchial, nasal, cutaneous, or conjunctival, although guidelines agree that the oral route is preferred whenever possible. The graded challenge is commenced with a small dose of the medication, which is gradually increased at 30-minute to 2 hours intervals provided no adverse response occurs after previous dose. The procedure is continued until the required dose; the total daily dose is reached. Placebo-controlled drug challenge may also be performed. Drug challenge should be performed in specialized centers with the highest safety conditions, with emergency resuscitative equipment and trained staff, to manage serious reactions in time [3], [5], [11], [13].

Figure 2: We summarize the suggested methodology for investigating drug hypersensitivity based on our publication, "Gyógyszerallergia: nemzetközi konszenzus" [Drug allergy: international consensus] in the *Hungarian Journal of Dermatology and Venereology*



1.3. 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. The primary infection appears more often in children, adolescents, and young adults [14]. Symptoms start with a prodromal phase that includes subfebrility, malaise, fatigue, headache, arthralgia, and myalgia, just like any common upper respiratory tract infection [15]. The classic features, fever, tonsillopharyngitis, lymphadenopathy, leukocytosis, and hepatosplenomegaly, are helpful in differentiation from bacterial infection. 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 [16]. 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 [15]–[18].

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, secretion of IgE and IgG4 by B-cells accompanies the reaction, connecting the delayed reaction to an immediate type I reaction [17].

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 with a prevalence of 3-6% among men and 1-2% among women in western developed countries. Serum urate testing is remarkably useful, although hyperuricemia alone is not sufficient for the diagnosis of gout [19]–[21]. There is evidence that hyperuricemia may, not independently, modestly increase the risk of coronary heart disease [22]. Other investigations found hyperuricemia to be an independent risk factor for coronary and heart diseases, heart failure, stroke, and cardiovascular death [23]–[25]. Studies have shown the potential cardioprotective effect of allopurinol therapy in decreasing the occurrence of acute cardiovascular events in gout and diabetes [26], [27].

Serum uric acid level need to be under 360 μmol/L (6 mg/dl) for all patients on urate-lowering therapy and a lower target, as 300 μmol/L (5mg/dl) is recommended for patients with severe arthritis. There is still no definitive standpoint on the medical treatment of asymptomatic hyperuricemia, diet adjustment, and lifestyle management can be more beneficial among patients with hyperuricemia without evidence of monosodium urate crystal deposition or gout. Among the urate-lowering drugs, the xanthine oxidase inhibitors (e.g.: allopurinol, febuxostat), drugs for inhibiting urate production, are widely used. Use of the allopurinol became prominent by the year 2005, in line with the investigation of its cardioprotective effect. The dosing recommendation support the "start low, go slow" treatment with appropriate monitoring of serum urate level, and renal and liver function.[19], [20], [24], [28].

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 [29], [30]. 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 [31], [32].

A strong association between the HLA-B*58:01 allele and severe allopurinol-induced adverse reactions, such as generalized maculopapular exanthems, hypersensitivity syndrome/ drug epidermal necrolysis (SJS/TEN), have been reported. SJS/TEN association with HLA-B*58:01 was first reported for the Han Chinese population (with 100% negative predictive value) and later in other populations, with lower negative predictive value, including Caucasians and other Asian nations [33]–[35].

2. Aims

Previously published papers suggest that interactions of viruses and penicillin may predispose individuals to specific illnesses outcomes. It became an issue whether this phenomenon may lead to persistent, true drug hypersensitivity or it is just a temporary reaction. In the past, in general, it was believed that the morbilliform skin rash following antibiotic intake in patients with infectious mononucleosis is a transient reaction, not a true allergic reaction. Today it is generally accepted that viral infections enhance the risk of developing drug allergies, although the underlying mechanism is still not fully understood.

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.

Apart from viral infection and antibiotics, in the last ten years, we have recognized allopurinol, a widely used drug, to be highly associated with cutaneous adverse reactions. Allopurinol can induce severe cutaneous adverse reactions and life-threatening medical conditions with a high mortality rate and poor prognosis. Since allopurinol prevention became more general in our country, we found an increase in allopurinol hypersensitivity reactions in our region.

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

3.1. 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 [7].

***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) [36], [37].

***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) [38]. 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 [39], [40]. 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.

3.2. 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 workup 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 [5], [41], [42]. 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/ Drug Reaction with Eosinophilia and Systemic Symptoms (DHS/ DRESS) cases among severe cutaneous adverse drug reactions, we applied the RegiSCAR criteria for DRESS [7], [43], [44].

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 [45]–[47]. 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 testing. The histopathology examination was done at our dermatopathology unit as part of a routine examination. The applied methods were done as described in the reference [48] with optimizations made at our laboratory. 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 (Novocasta, NCL-L-CD1a-235 clone, 1:50 dilution), CD20 (Dako, CloneL26 M0755 clone, 1:200 dilution), CD34 (Dako, QBEN/10 M7165 clone, 1:200 dilution) CD45 (Dako, 2B11+PD7/26 clone, 1:100 dilution) and CD68/KP1 (Bio Care, CN033C clone, 1:400 dilution) helped us in immunohistopathology. Primary immunofluorescence assay was used to detect deposits of

different immunoglobulin isotypes, such as immunoglobulin A (Dako, rabbit anti-human IgA/FITC, 1:32 dilution), immunoglobulin G (Dako, rabbit anti-human IgG/FITC, 1:32 dilution) and immunoglobulin M (Dako, rabbit anti-human IgM/FITC, 1:32 dilution) and complement proteins, as complement component 3 (Dako, rabbit anti-human C3c/FITC, 1:32 dilution) 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 (QIAamp^{R} genomic DNA and RNA kits), then real-time polymerase chain reaction amplification and reverse hybridization using the PG5801 Detection Kit from the Pharmigene Inc.

Statistical analysis

R Studio software (R version 4.2.3) was used for statistical analysis. Categorical variables were evaluated with a statistical significance test used to analyze contingency tables; the Fischer's exact test. Subjects with a lack of allopurinol use were sorted randomly into the control group. The control and the test group had the same age-gender distribution. 37 study participants were sorted into each group, each with 19 female and 18 male participants, the mean age was 70 years among female participants and the mean age was 67 years among male participants in both groups. 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.

3.3. Ethical approval

The study was approved by the regional and national ethics committees. All patients have given their permission to use their clinical information and photographic material relating to the subject for educational publications intend for health professionals.

4. Results

4.1. Study I: Infectious mononucleosis and drug hypersensitivity

10 patients who were treated at our clinic between 2002 and 2012 (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 (Figure 3). In the case of Patient number 4 with progressive maculopapular rash skin biopsy was performed. 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 (Figure 4).

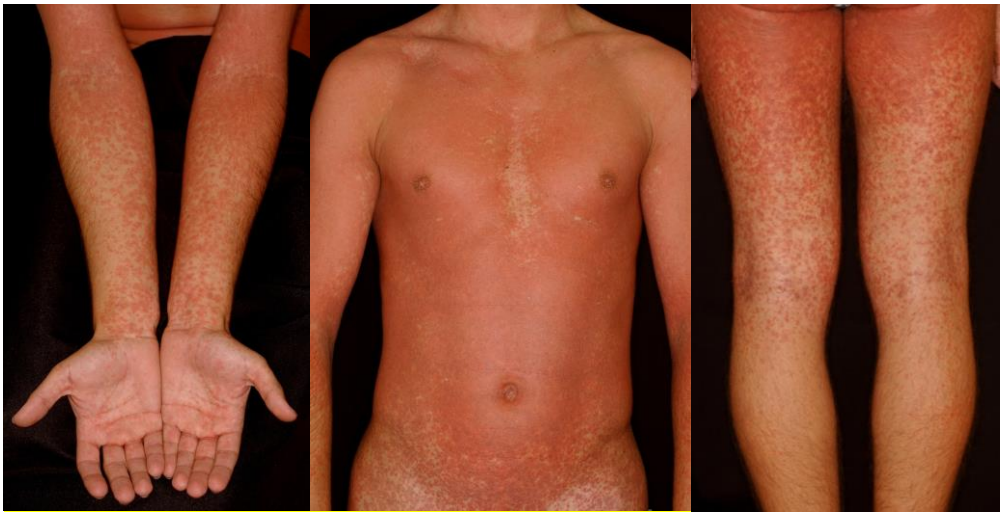


Figure 3: Amoxicillin rash in a patient with infectious mononucleosis (patient 4)

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.

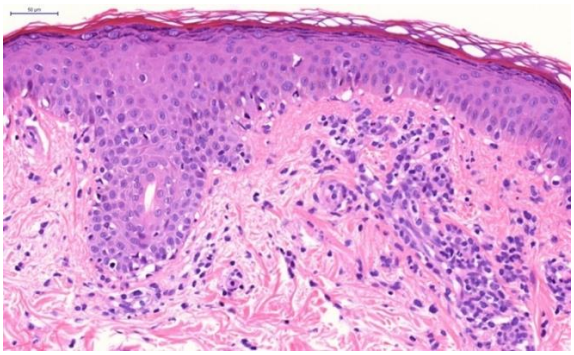


Figure 4: Histopathological image of Patient 4 with maculopapular rash (112x magnification)

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 (Figure 5). Patch tests were performed after negative prick and intracutaneous testing with negative results in the remaining two patients (Table 3).

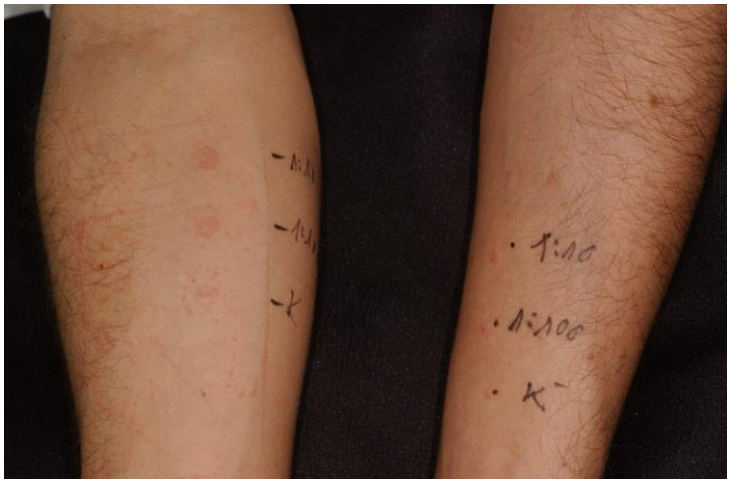


Figure 5: Positive cutaneous response (Penicillin allergenic determinants (DAP) ® test)

The *in vivo* cutaneous investigation was continued with intradermal testing if the prick tests resulted in a negative response at different dilutions. Skin tests were performed using penicillin's main antigens, the major determinant (PPL), and the minor determinant mix (MDM). Cutaneous tests were started with the major determinant; the negative control was a saline solution. In this case, we recognized positive skin reaction to MDM at 1:100 and 1:10 dilutions, which verified the development of sensitization (patient 4).

Patient	Age (years)	Gender	Culprit drug	LTT results	Prick test results	Intradermal test results	Patch test results
1	15		amoxicillin/clavulanic acid	Negative	Negative	**PPL 1:100 and 1:10 Positive**	Not performed
2	19		amoxicillin	Negative	Negative	**MDM undiluted Positive**	Not performed
3	29		amoxicillin/clavulanic acid	Negative	Negative	**PPL 1:10 Positive**	Not performed
4	23		amoxicillin/clavulanic acid	Negative	Negative	**MDM 1:100 and 1:10 Positive**	Not performed
5	35		amoxicillin/clavulanic acid	Negative	Negative	Negative	Negative
6	24		amoxicillin/clavulanic acid	Negative	Negative	Negative	Negative
7	21		amoxicillin	Negative	Not performed	Not performed	Not performed
8	20		amoxicillin/clavulanic acid	Negative	Not performed	Not performed	Not performed
9	16		amoxicillin/clavulanic acid	Negative	Not performed	Not performed	Not performed
10	27		amoxicillin/clavulanic acid; cefixime	Positive: cefixime	Not performed	Not performed	Not performed

Table 3: True sensitization to amoxicillin examined by *in vitro* and *in vivo* procedures

PPL: major determinant: benzylpenicilloyl poly-L-lysine. MDM: minor determinant mix: sodium benzylpenicillin, benzylpenicilloic acid, sodium benzylpenicilloate. Bold text: Verified sensitization to penicillin. Tests were done in the following chronology: LTT → Prick test (non-diluted PPL) → Intradermal test (1:100 dilutions of PPL, 1:10 dilution of PPL, non-diluted PPL) → Prick test (non-diluted MDM) → Intradermal test (1:100 dilution of MDM, 1:10 dilution of MDM, non-diluted MDM) → Patch test (culprit drug).

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 [18].

4.2. 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. The 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 ($p = 0.0007$, OR =7.59), beta-blockers ($p = 0.002$, OR = 4.87), diuretics ($p = 1.19*10^{-8}$, OR = 28.73), proton-pump inhibitors ($p = 0.008$, OR = 4.18), and thrombocyte aggregation inhibitors ($p = 7.69*10^{-5}$, OR = 9.07) was significantly high among the studied group (Table 4).

	Percentage of patients taking various drugs (%)							
	ACE–I	**Benzo-diazepines**	**Beta-blockers**	**Diuretics**	**HMG CoA R-I**	**NSAID drugs**	**PPIs**	**Anti-platelet drugs**
Vasculitis	50%	50%	50%	100%	50%	0%	0%	50%
MPE	63%	50%	75%	88%	50%	25%	44%	50%
ED	50%	0%	100%	100%	50%	50%	50%	100%
AGEP	0%	0%	100%	100%	100%	0%	100%	100%
DRESS	39%	54%	77%	92%	54%	62%	77%	62%
SJS	50%	50%	0%	100%	0%	50%	0%	50%
SJS/TEN	0%	100%	100%	100%	0%	0%	100%	100%

Table 4: Risk factors for allopurinol-associated hypersensitivity: co-medication

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 ($p = 0.0095$, OR = 5.99), renal failure ($p = 2.68*10^{-5}$, OR = Infinity), and vascular disorders ($p = 3.70*10^{-6}$, OR= 12.77) was significantly high among the studied group (Table 5).

	Percentage of patients with various comorbidities					
	Diabetes	**Heart failure**	**Hypertension**	**Liver disease**	**Renal failure**	**Vascular disorders**
Vasculitis	100%	50%	100%	0%	0%	50%
MPE	25%	31%	81%	19%	25%	50%
ED	0%	100%	100%	0%	50%	100%
AGEP	0%	0%	100%	0%	0%	100%
DRESS	31%	39%	100%	0%	62%	85%
SJS	50%	50%	100%	0%	0%	100%
SJS/TEN	0%	0%	100%	0%	100%	0%

Table 5: Risk factors for allopurinol associated-hypersensitivity: co-morbidity

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 did not have exact information in 9 cases (Table 6). 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 (Fig. 7).

Cutaneous manifestations were accompanied by different systemic signs. See detailed information on clinical characteristics of allopurinol-induced hypersensitivity reactions in Table 7.

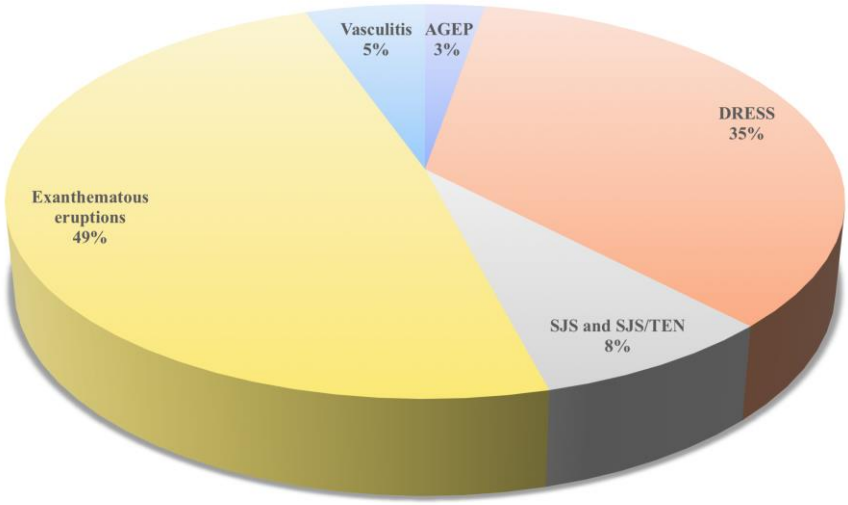


Figure 6: Distribution of allopurinol-induced adverse drug reactions among our patients

Table 6: Allopurinol-induced adverse reactions

Patient #	Age/ Gender (years)	Allopurinol (weeks)	Adverse drug reactions	LTT results	Renal function	Diuretics
1	60/ M	1	vasculitis	negative	normal	amiloride, HCT
2	65/ M	N/A	vasculitis	positive	normal	IND, FUR
3	48/ M	3	MPE	negative	normal	no
4	48/ M	2	MPE	negative	normal	no
5	52/ M	2	MPE	negative	impaired	clopamide, FUR
6	63/ M	3	MPE	negative	normal	IND
7	67/ M	4	MPE	negative	normal	FUR, SPL
8	68/ F	4	MPE	positive	impaired	HCT, SPL
9	73/ F	N/A	MPE	negative	impaired	SPL
10	75/ F	4	MPE	negative	impaired	FUR, HCT
11	77/ F	4	MPE	negative	impaired	FUR
12	79/ M	8	MPE	negative	N/A	IND, FUR
13	80/ F	1	MPE	negative	impaired	clopamide, FUR
14	80/ F	N/A	MPE	positive	normal	amiloride, HCT, FUR, clopamide,
15	82/ M	N/A	MPE	negative	impaired	HCT
16	85/ F	1	MPE	negative	normal	IND
17	86/ M	3	MPE	negative	impaired	HCT, FUR, SPL
18	87/ M	8	MPE	negative	normal	SPL
19	61/ M	6	ED	negative	impaired	clopamide, etacrynic acid, FUR, SPL
20	71/ F	4	ED	negative	impaired	FUR, HCT
21	71/ M	3	AGEP	negative	impaired	HCT, FUR
22	46/ M	5	DRESS	negative	impaired	IND, FUR, SPL
23	51/ F	4	DRESS	negative	normal	HCT
24	52/ F	N/A	DRESS	negative	impaired	FUR
25	55/ F	4	DRESS	negative	impaired	no
26	60/ M	4	DRESS	negative	impaired	FUR
27	66/ F	3	DRESS	negative	impaired	HCT
28	67/ F	4	DRESS	positive	impaired	etacrynic acid, FUR
29	71/ F	6	DRESS	negative	impaired	HCT
30	73/ F	4	DRESS	negative	N/A	HCT, FUR, SPL
31	76/ M	N/A	DRESS	negative	impaired	HCT
32	76/ F	1	DRESS	negative	impaired	FUR
33	81/ F	N/A	DRESS	negative	impaired	clopamide, HCT, FUR
34	90/ M	N/A	DRESS	negative	impaired	clopamide, FUR
35	66/ M	2	SJS	negative	normal	IND
36	71/ F	N/A	SJS	negative	impaired	HCT
37	65/ F	3	SJS/TEN	negative	impaired	HCT, IND

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 direct immunofluorescence (DIF) testing among our patients. See important histologic patterns in association with cutaneous symptoms in Fig. 7 and Table 7.

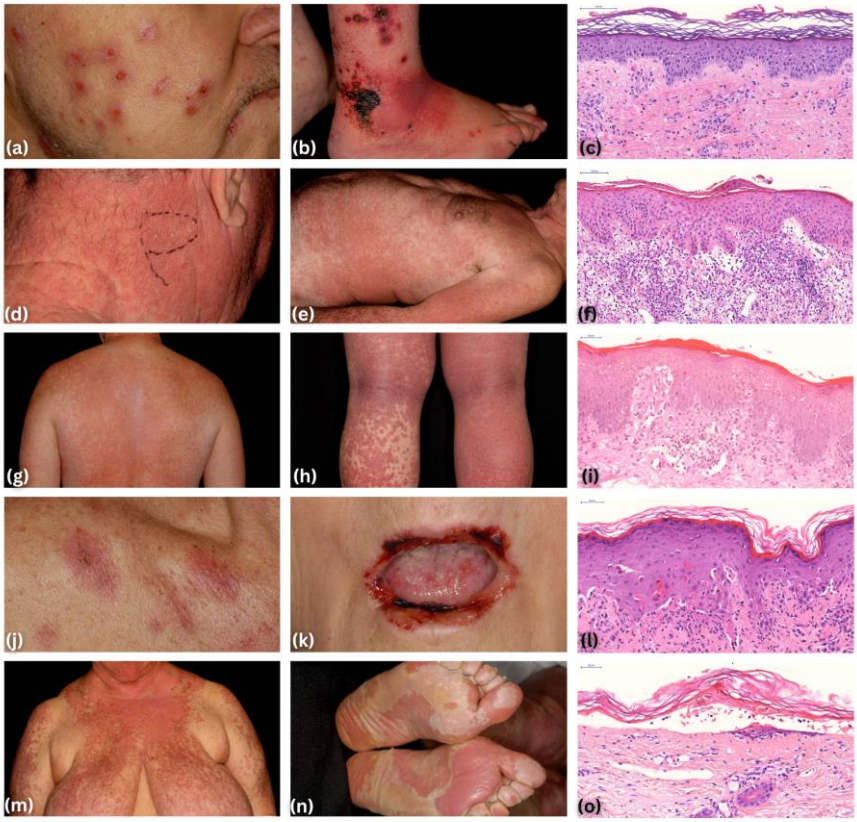


Figure 7: Cutaneous and histological signs of allopurinol-induced adverse reactions

(a)-(b) Clinical features of a patient with *vasculitis*. *(c)* showing the histopathological image of a vasculitis patient with dermal perivascular infiltrate, incipient leukocytoclasis, and several eosinophils (112x magnification). *(d)-(e)* Clinical features of a patient with *AGEP*. *(f)* showing the histopathological image of an AGEP patient with acanthosis and spongiosis in the epidermis along with parakeratosis and incipient pustule formation, subepithelial fissure, dermal mononuclear infiltration, and scattered eosinophils (112x magnification). *(g)-(h)* Clinical features of a patient with *DRESS*. *(i)* showing the histopathological image of a DRESS patient with acanthosis and spongiosis in the epidermis with an interface mononuclear cell infiltration, and scattered eosinophils (112x magnification). *(j)-(k)* Clinical features of a patient with *SJS*. *(l)* showing the histopathological image of an SJS patient with interface dermatitis with several cytoid bodies (112x magnification). *(m)-(n)* Clinical features of a patient with *SJS/TEN overlap*. *(o)* showing the histopathological image of an SJS/TEN patient with a severe detachment of the necrotic epidermis (200x magnification). Histologic sections were stained with hematoxylin-eosin and studied by a brightfield microscope.

Table 7: Clinical and histologic characteristics of allopurinol hypersensitivity

	Vasculitis	AGEP	ED	MPE	DRESS	SJS	SJS/TEN
Skin lesions	palpable purpura on face and extremities	generalized, small, multiple, non-follicular, and sterile pustules on diffuse erythema	bright red, thickened skin	generalized, symmetric, discrete and/or confluent macules and papules; palpable purpura-like lesions on lower extremities	generalized, symmetric, discrete, and/or confluent macules and papules on diffuse erythema	large confluent macules and target-like lesions on the trunk and face; eyelid edema; 1-9% epidermal detachment	generalized, symmetric macules and papules (color tones of purple); 10-30% epidermal detachment
Mucous membranes	without involvement	without involvement	oral mucosa: ± enanthem and/or erosion	oral mucosa: ± enanthem and/or erosion	oral mucosa: enanthem and/or petechial hemorrhage	oral, genital mucosa: enanthem, erosion; conjunctivitis keratitis	oral, genital mucosa: enanthem, erosion; conjunctivitis keratitis
Other symptoms	pruritus pain shivers fever malaise albuminuria elevated erythrocyte sedimen-tation rate	pruritus shivers fever	pruritus pain shivers fever malaise	pruritus ± burning sensation shivers ± fever ± malaise	pruritus burning sensation fever malaise ± nausea hepatitis nephritis lymphadeno-pathy	pain burn sensation (skin and mucosa) tenderness fever malaise anxiety arthralgia; increased serum urate	intense pain burn sensation (skin and mucosa) tenderness fever malaise anxiety arthralgia; increased serum urate
Hemogram	lymphopenia; normal platelet count	leukocytosis neutrophilia	anemia leukocytosis lymphopenia neutrophilia	lymphopenia eosinophilia	leukocytosis lymphopenia eosinophilia; atypical lymphocytes	anemia lymphopenia ± neutro-penia	anemia lymphopenia neutropenia
Histology	eosinophil-rich infiltrate around and within the walls of small dermal vessels; signs of vacuolar degeneration of keratinocytes at DEJ	subcorneal and intraepiderm al pustules; interface dermatitis with eosinophils and neutrophils	epidermal spongiosis; hydropic degeneration of the basal cell layer; lymphohistio -cytic infiltrate (eosinophils, neutrophils) around and within the walls of small dermal vessels	epidermis: acanthosis, spongiosis, individual and/or confluent necrosis of basal keratinocytes dermis: papillary dermal edema, spf. perivascular lymphohistio -cytic infiltrate with eosinophils, neutrophils	epidermis: spongiosis, focal parakeratosis vesicle formation, individual and/or confluent necrosis of basal keratinocytes dermis: spf. perivascular lymphohistio -cytic infiltrate with eosinophils, neutrophils	epidermal spongiosis; vacuolar interface dermatitis; individual and/or confluent necrosis of basal keratinocytes spf.perivasc. lymphohistio -cytic infiltrate, some eosinophils; extravasated erythrocytes	superficial perivascular lymphohistio -cytic infiltrate with some eosinophils; extravasated erythrocytes; vacuolar interface dermatitis; satellite cell necrosis; full-thickness epidermal necrosis
Direct IF testing	IgM and C3 deposits in the walls of small dermal vessels/ DEJ	no immunopath ologic discrepancy	C3 deposits in the walls of small dermal vessels/ DEJ	C3 deposits in the walls of small dermal vessels/ DEJ	C3 deposits in the walls of small dermal vessels/ DEJ	C3 deposits in the walls of small dermal vessels/ DEJ	C3 deposits in the walls of small dermal vessels/ DEJ

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

The development of skin rash following amoxicillin intake in patients with infectious mononucleosis is quite frequent among beta-lactam-induced adverse drug reactions [15]. These eruptions are maculopapular exanthems. The exact mechanism behind these eruptions is unclear. It is not well explained yet, whether a true allergic drug reaction, virus-dependent rash, or transient loss of drug tolerance due to the virus is responsible for the symptoms. The rash may be due to the viral infection itself, the incidence of skin eruption development in acute infectious mononucleosis is 4.2-13% without drug intake, but often these patients are put on antibiotics, frequently amoxicillin, and the rash appears a few days after the initiation of the antibiotic therapy. Following amoxicillin intake within acute infectious mononucleosis the incidence of skin reactions ranges between 27.8% and 69%, while in children, morbilliform skin eruptions nearly always develop following amoxicillin intake within acute infectious mononucleosis [14]–[18], [49].

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 [17], [18]. 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 to the culprit drugs could answer this question. We cannot explain the negative results of LTT in all of these cases to penicillin. It may be that our method could be improved, but it may be that this group of patients is not one in which LTT could work. We do get positive LTT results for penicillin, 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 patients with infectious mononucleosis patients suffering from amoxicillin rash.

With this investigation we would like to further emphasize the importance of allergy examination in patients with generalized skin lesions after penicillin intake in infectious mononucleosis, to verify whether true sensitization developed.

Study II: Hyperuricemia and drug hypersensitivity

Monitoring serum urate levels is suggested in patients with high cardiovascular risk, although there is no definitive recommendation on asymptomatic hyperuricemia drug treatment. If measuring serum uric acid levels above 360 μmol/L twice in a row (at least two weeks apart), recent studies suggest a "three-step method", first is to search and eliminate external causes and underlying diseases of elevated serum urate levels, then the help of changed lifestyle and appropriate diet urate lowering should be achieved. If the patient has cardiovascular disease, gout, and/or nephrolithiasis or the risk for developing these disorders it is advised to lower the serum uric acid level and keep it under 360 μmol/L with the help of urate-lowering drugs [23], [24], [50], [51].

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 reduction of the urate level [23]–[25], [52]. 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 prognosis [29], [30], [53]. Since allopurinol prevention became more generalized in our country, we found an increase in 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 developing allopurinol-associated adverse reactions. Administration of benzodiazepines, beta-blockers, diuretics, proton-pump inhibitors, and thrombocyte aggregation inhibitors 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 [54], [55]. 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 [56], [57]. 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 populations, while a relevant but weaker association was found in patients of Causasian 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 Caucasian population DRESS was also connected to this variant similar to our patient [33], [35]. 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 even in patients without HLA-B*58:01 allele association [58], [59].

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 [60]. 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. Few studies reported previously that oxypurinol-specific lymphocyte response predominates the allopurinol reaction, specifically in patients with the HLA-B*58:01 allele. The high drug concentration and rapid conversion of allopurinol to oxypurinol *in vivo* may have a major role in the adverse drug reaction. No cross-reactivity has been shown between allopurinol and oxypurinol T cell lines [56]. The role of oxypurinol-specific T-cells in allopurinol-induced hypersensitivity reactions could explain the lack of LTT positivity in our patients.

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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