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REVIEW ARTICLE

Cutaneous allergic drug reactions: update on pathophysiology, diagnostic procedures and differential diagnosis

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Abstract

Important changes in the understanding and management of drug hypersensitivity reactions during the last years result from the increasing importance of biologics in medical practice, which differ in their spectrum of adverse drug reactions (ADRs) from the classical covalent drugs. With regard to covalent drugs, ampicillin and amoxicillin as well as clavulanic acid play an increasing role among ADRs to betalactam antibiotics. Fluoroquinolones are mainly the cause of anaphylactic and photosensitivity reactions. Especially in allergic reactions to NSAIDs, pseudoallergic reactions should be considered in the differential diagnosis. In opposite to the main cutaneous allergic drug reactions such as urticaria or maculopapular skin rash, in which antibiotics are the main culprits, in severe drug allergic reactions such as SJS (Stevens-Johnson Syndrome), TEN (Toxic Epidermal Necrolysis), or DRESS (Drug Reaction with Eosinophilia and Systemic Symptoms) Syndrome, compounds like allopurinol and anticonvulsants are the main causes. Similar mutations in the IL36R gene, which were found in both patients with an AGEP (Acute Generalized Exanthematous Pustulosis) and pustular psoriasis, make the differential diagnosis more difficult and raise the question whether there is a difference between these diseases or whether AGEP is not just a drug induced pustular psoriasis. Finally, some special aspects of side effects of biologics and targeted therapies respectively are discussed.

Keywords

Antibiotics, biologics, SCARs, skin rash, urticaria

History

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Introduction and epidemiology

The skin is a signaling organ for adverse drug reactions (ADRs) and it has been considered that about 3–7% of all hospital admission or referrals from primary care physicians are made due to ADRs of the skin^{1,2}. Besides the skin, liver and blood cells are the most frequently affected targets³.

The reactions which are determined solely by the pharmacological profile of administered drugs (A- or on-target reactions) should be distinguished from those in which in addition to the pharmacological profile, individual factors of the patient also play a role (off-target or B-reactions) (Table 1). About 30% of these B-reactions to small molecular weight compounds – also known as covalent drugs – are allergic ADRs⁴. Risk factors for these reactions are dependent from drug-related factors as well as from patient-related factors. Examples are given in Table 2. With regard to biologics, such a definition, in the most cases, is more difficult, because medical conditions, which are associated with allergic reactions to biologics, can be also explained by the pharmacological profile of these substances.

Therefore, we would like to focus first on the allergic reactions to “covalent drugs”, followed by some aspects of the reactions to biologics.

Pathogenesis

The basis for the allergic drug reactions is a specific sensitization of the immune system to small molecular substances, that lead to the formation of specific antibodies (e.g. IgE antibodies) or specifically reactive T-lymphocytes. The skin is a preferred target organ for allergies to small molecular substances, which besides multiple signs and symptoms of different drug-induced skin rashes may also present as allergic contact dermatitis⁵. One of the reasons for this is the property of the skin to induce hypersensitivity reactions after sensitization and not primarily the tolerance, as the immune system of the gastro-intestinal tract does⁶. It has been shown in animal studies that sensitization to cyanates or platinum salts resulting in IgE-dependent asthma could be only reached by primary sensitization through the skin⁷.

It is generally accepted and known as the hapten hypothesis that small molecular weight compounds have to bind to high molecular ones, such as peptides or proteins, in order to become antigenic⁸. There is a strong evidence that most drug allergies are caused by reactive metabolites of the drug rather than the parent drug itself^{9,8}. Xenobiotica metabolizing enzymes are present in extrahepatic organs such as the skin, including keratinocytes and antigen-presenting, immunocompetent cells such as Langerhans cells^{10–13}. In addition, the associations between cytochrome P 450 polymorphisms and drug allergic reactions (e.g. to phenytoin) have been shown¹⁴.

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Table 1. Classification of adverse drug reactions (ADR).

Type A: Pharmacologically explainable reactions	
-	Drug overdoses
-	Side effects
-	Drug interactions
Type B: Reactions to drugs, depending on individual risk factors	
-	<i>Drug allergy</i> : Immunologically explainable reactions
-	<i>Pseudoallergic reactions</i> : The same clinical manifestation as in allergic reactions, but pathophysiologically there are no sensitization and immunological specificity
-	<i>Drug intolerance</i> : Adverse drug reaction, which corresponds with the pharmacological characteristics of the drug, but occurs at unexpectedly low doses of the substance and cannot be explained by individual patient-related factors, including particularities of metabolism, pharmacokinetics or bioavailability
-	<i>Drug idiosyncrasy</i> : Adverse drug reaction, which corresponds with the pharmacological characteristics of the drug, but occurs at unexpectedly low doses of the substance and can be explained by individual patient-related factors, including particularities of metabolism, pharmacokinetics or bioavailability

Table 2. Risk factors for allergic drug reactions.

Patient-related factors	
Age	Young adults > children/elderly patients
Gender	Women > men
Polymorphisms	HLA, drug-metabolizing enzymes
Viral infection	HIV, herpes infections
Cross-reactions	A history of allergic reaction to chemically related drugs or allergic contact dermatitis to chemically related substances
Drug-related factors	
Molecular size	High-molecular substances or good binding haptens
Application	Topical > intravenous/intramuscular > oral
Dose	Frequent and intermittent > single dose

The metabolism of drugs to highly reactive intermediates may influence the pathogenesis of allergic drug reactions twofold. Firstly, the formation of highly reactive products increases the ability of the drug to bind to high-molecular weight substances. Secondly, these highly reactive substances represent a “danger signal”, which can cause an increased risk of sensitization^{6,15}. Also different diseases – in particular infections – create an immunological environment that favors sensitization to drugs. This theory is supported by the observation, that at least the most common ADRs – allergic urticaria and maculopapular rash – in most cases are triggered by antibiotics, which are administrated in a disease that itself activates the immune system by the dangerous infection constellation³.

The pharmacological interaction hypothesis (p-i) proposed that immunological receptors for drugs have already existed on T-lymphocytes prior to the administration of the drug as a kind of molecular mimicry caused, e.g. by viral or bacterial infections¹⁶. This concept is supported by several HLA associations with allergic drug reactions (Table 3). Meanwhile, about 40 HLA-associated ADRs for special drugs and populations have been described^{17,18}. A typical example for this is the carbamazepine-induced bullous drug reaction, Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) associated with the HLA-B*1502 allele in Chinese¹⁹. It has been shown that MHC proteins of this HLA

Table 3. HLA association with adverse drug reactions^{50,71}.

Drug	Adverse drug reaction	Ethnic background	Allele
Allopurinol	SJS/TEN	Han Chinese Japanese Thai Caucasian	HLA-B*5801
Carbamazepin	DRESS	Han Chinese	HLA-B*5801
	SJS/TEN	Han Chinese ^{a/b} Thai ^b Malaysians	HLA-B*1502
		Indians	
		Japanese	HLA-B*1511
Abacavir		Caucasian	HLA-A*3101
Nevirapin	Rash and hepatitis	Caucasian	HLA-B*5701
	DRESS	Caucasian	HLA-DRB1*0101
		Sardinian	HLA-Cw8-B14 (haplotype)
			HLA-Cw8
	DRESS (rash only)	Thai	HLA-B*3505
	DRESS (with rash)	Caucasian	HLA-B*3501
Lumiracoxib	Hepatitis	Caucasian	HLA-DRB1*1501
Flucloxacillin	Hepatitis	Caucasian	HLA-B*5701

^aAlso oxcarbazepin.
^bAlso phenytoin.

allele can particularly favorable bind carbamazepine¹⁹. However, only some patients with this HLA allele develop on administration of carbamazepine SJS. The carbamazepine-reactive T-lymphocytes from these patients have been cloned and it has been shown that the patients who had this HLA-allele and developed a SJS upon carbamazepine, had also, in almost all cases, an identical T cell receptor¹⁹. This means that the presence of specific HLA allele and particularly of MHC class I molecule in combination with a defined T cell receptor not only causes allergic sensitization upon administration of carbamazepine, but necessarily leads to the clinical picture of SJS or TEN, however not to other allergic reactions, such as anaphylaxis. These findings have already led to the restriction in prescribing of carbamazepine to Asians that may be performed only after confirmation of the absence of the HLA-B*1502 allele. Nevertheless, in some cases such binding of the drug to the MHC-proteins may not occur directly and therefore require a metabolic process. An example for this is allopurinol. Its oxidation product, oxypurinol, acts as a ligand of MHC-molecule; thus this metabolic product should be used in diagnostic investigations instead of allopurinol^{20–22}.

The p-i-hypothesis is concluded from studies with polyclonal or monoclonal activation of T-lymphocytes by a drug, which induced a drug allergic reaction in a patient who was sensitized to this drug and experienced a drug allergic reaction. Therefore, this hypothesis is based on the assumption, that the compound which activates the lymphocytes is also the compound which induces the signs and symptoms of the allergic drug reaction¹⁶. However, the results of a recently developed animal model of nevirapine-induced severe drug allergic reactions ruled out that this assumption is wrong⁹. Nevirapine is used for the treatment of HIV and is well-known for triggering severe bullous skin reactions in up to 5% of all patients. Using Brown Norway rats as an animal model for this disease, it became possible to investigate the pathogenesis

of these allergic drug reactions. These studies showed that unlike the original substance nevirapine, its metabolite 12-hydroxy-nevirapine is able to sensitize the rats and then trigger a skin reaction after sulfation⁶. However, *in vitro* analysis of the T-lymphocytes of these rats showed only an activation induced by nevirapine itself and no activation after incubation with 12-hydroxy-nevirapine. In addition, the reactive benzylic sulfate metabolite of nevirapine, which is involved in the skin rash by nevirapine, may also induce an inflammasome activation⁹. The inflammasome belongs to the NOD-like receptor family and induces upon activation proinflammatory cytokines such as interleukin1 and interleukin 18.

The observation that parent compounds can activate T-lymphocytes in the lymphocyte transformation test (LTT), although different metabolites of the same drug cause sign and symptoms of the drug allergic reaction, exists also for isoniazid and sulfamethoxazole⁹. In the LTT, lymphocytes are able to recognize both the original substance sulfamethoxazole, as well as its metabolites. Interestingly, T-lymphocytes from patients with cystic fibrosis sensitized to sulfonamides consist only of clones which are reactive to one of the metabolites or to one of the metabolites and the original substance. In comparison to this finding, patients without cystic fibrosis sensitized to sulfonamides also possess T-cell clones that react solely to the original compound. This phenomenon could be explained by the increased oxygen stress of the cells in patients with cystic fibrosis, and it demonstrates the importance of such oxygen stress for the pathogenesis of allergic reactions to drugs²³. Also in the model of allergic reaction to sulfamethoxazole using human keratinocytes was demonstrated that in the keratinocytes of sensitized patients occur a simultaneous staining of sulfamethoxazole-metabolites and MHC-I structures, which means that not the original substance, but the activated metabolite binds to the MHC-I structures acting as the possible signal for triggering of the allergic skin reaction²⁴. Even cloned lesional T-lymphocytes of patients with allergic sensitization to sulfamethoxazole led to T-cell clones which could be activated by sulfamethoxazole only after the addition of sulfamethoxazole metabolizing microsomes^{24,25}.

To summarize, the pathophysiology of allergic drug reactions is significantly affected by:

- (1) A proinflammatory state of immunocompetent cells which promotes sensitization to drugs.
- (2) The binding ability of the drug or its metabolites to structures such as T-cell receptors or proteins on antigen presenting cells and/or its ability to activate the inflammasome.
- (3) Oxidative stress, so that the metabolism of drugs leads to the formation of the sensitizing antigen and on the other hand the metabolism itself may be a trigger of the oxidative stress and represent a danger signal, which promotes allergic sensitization.

Clinical signs and symptoms of allergic drug reactions

Most allergic drug reactions precipitate as a maculopapular rash or urticaria (Figure 1). Urticaria, angioedema and



Figure 1. Clinical pictures of allergic drug reactions. AGEP: Acute Generalized Exanthematous Pustulosis; DRESS: Drug Reaction with Eosinophilia and Systemic Symptoms.

anaphylactic shock require a previous allergic sensitization, which leads to the formation of specific IgE antibodies. Differential diagnosis includes pseudoallergic reactions which present with the same signs and symptoms without previous specific sensitization. The examples for the pseudoallergic reactions are:

- Analgesic intolerance,
- Angioedema caused by ACE inhibitors,
- Activation of mast cells through a single receptor, MrgprX2.

Besides antibiotics, NSAIDs are the most common cause of allergic and pseudoallergic reactions to drugs. Cutaneous immediate type reactions to analgesics can be distinguished as at least three different pathophysiological reactions^{26,27}:

- Classic IgE-dependent reactions, mostly to metamizole or other pyrazolone derivatives, but also to paracetamol, ibuprofen or diclofenac.
- Pseudoallergic reactions to acetylsalicylic acid and other COX-1 inhibitors. The causes of these reactions are very likely different polymorphisms of the arachidonic acid metabolizing enzymes or receptors for various arachidonic acid metabolites. The diversity of these polymorphisms makes it difficult to use this observation in clinical diagnostics.
- NSAIDs are able to augment IgE-dependent allergic reactions, as it has been demonstrated in wheat-dependent exercise-induced anaphylaxis (WDEIA) caused by sensitization to omega-5-gliadin²⁸. There is also a polymorphism that has an augmenting effect on IgE-dependent reactions and correlates with the manifestation of a NSAID-related anaphylaxis²⁹.

In addition, it can be differentiated based on whether the skin or the lung is the main target organ which leads to the classification outlined in Figure 2.

Moreover, recent research suggests a further mechanism of pseudoallergic reactions. MrgprX2, a mast cell specific receptor was identified to be crucial for pseudoallergic drug reactions. Neuromuscular blocking drugs, including tubocurarine and atracurium, and fluoroquinolone family of antibiotics (e.g. ciprofloxacin) are able to activate mast cells via binding at MrgprX2 evoking release of histamine³⁰.

Maculopapular drug eruptions, fixed drug eruptions (FDE) and most of the photoallergic drug reactions are delayed-type drug hypersensitivity reactions. About 90% of all allergic

Type of reaction	Signs and symptoms	Timing	Underlying disease	Cross-reactivity	Allergy +/-	Putative mechanism
NERD: NSAID-exacerbated respiratory disease	Bronchial obstruction, dyspnoea, nasal congestion, rhinorrhoea	immediate	Asthma/Rhinitis	+	-	COX1 inhibition
NECD: NSAID exacerbated cutaneous disease	Wheal and/ or angioedema	immediate	Chronic urticaria	+	-	COX1 inhibition
NINA: NSAID induced urticaria/ angioedema	Wheal and/ or angioedema	immediate	No underlying disease	+	-	COX1 inhibition
SNIUAA: Single NSAID-induced urticaria/angioedema/ anaphylaxis	Wheal and/ or angioedema	immediate	No underlying disease	-	+	IgE dependent
SNIDR: Single NSAID-induced delayed reactions	Various symptoms including FDR, SJS, TEN, nephritis	delayed	No underlying disease	-	+	T-cell dependent

Adapted from Dona et al., *J Investig Allergol Clin Immunol* 2014; 24(3): 143-53

Figure 2. Classification of reactions induced by NSAIDs.

delayed-type reactions are maculopapular drug eruptions, which usually occur 1–2 weeks after initiation of therapy. This fact is important for the anamnestic survey of such kinds of reactions. Important triggers of these reactions are CD4+ T-lymphocytes in contrast to the severe allergic delayed-type drug reactions, especially TEN, in which the main players are CD8+ cytotoxic T-lymphocytes^{31,32}. Fixed drug eruptions are also mediated by CD8+ T-lymphocytes, which are localized in the skin areas affected by the disease³³. FDE can occasionally affect several skin areas and is therefore difficult to distinguish from SJS and even TEN. Even in the first paper describing TEN (or Lyell syndrome) there were presented several patients which were suffering from such multiple FDE, as was stated later by Lyell himself³⁴.

The most severe skin drug reactions which should be aware of are SJS and TEN. They are characterized by:

- (1) At least two mucosal areas affected,
- (2) In the case of SJS not more than 10% of skin surface and in TEN more than 30% of skin surface affected,
- (3) In case if 10–30% of skin surface are affected, one would speak about SJS/TEN overlap.

For prognostic evaluation of TEN exists a special severity-of-illness score for toxic epidermal necrolysis (SCORTEN) (Figure 3)¹⁷. The most common culprit drugs for this severe skin reaction are anticonvulsants and allopurinol, but also antibiotics may be a cause for such clinical condition³⁴.

As it has been already mentioned, the investigation of lesional T-lymphocytes in bullous drug reactions showed cytotoxic CD8+ T-lymphocytes³². Meanwhile, it could be shown that these cytotoxic T-cells induce the destruction of keratinocytes². In the pathophysiology of this process, a direct binding of the cytotoxic T-cells to the affected keratinocytes may occur, beside a destruction of keratinocytes by soluble mediators, such as granulysin³⁵. Another pathophysiologically relevant factor is the tumor necrosis factor-related

apoptosis-inducing ligand (TRAIL)^{36,37}. Further investigations have now shown that a specific apoptosis process, also known as necroptosis, plays a central role and activates a pathway, which leads to the destruction of keratinocytes in SJS/TEN³⁸.

DIHS (Drug immune hypersensitivity syndrome)/DRESS (drug related eosinophilia systemic symptoms) is another severe drug reaction, which usually manifests itself on the skin with a maculopapular drug eruption, but is characterized by the systemic inflammatory response. It was initially considered to be induced only by anticonvulsants, but also other drugs have been proved to cause this reaction. A longer latency period of at least 4–6 weeks after initiation of therapy with the culprit drug is usually observed in DIHS, compared to about 10–14 days in the case of other skin rashes. The DRESS defined by seven features:

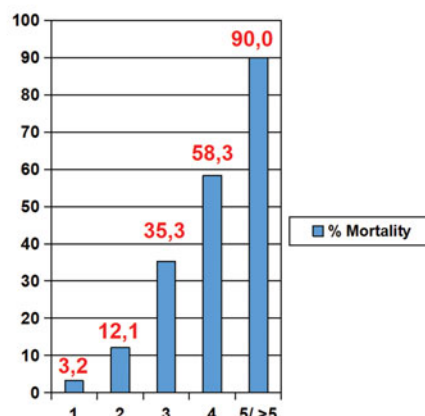
- (1) A maculopapular drug eruption,
- (2) The occurring clinical symptoms persist for several weeks, even when the drug is discontinued,
- (3) Patient develops fever,
- (4) Elevated hepatic transaminases,
- (5) Leukocytosis,
- (6) Eosinophilia,
- (7) Lymphadenopathy and about 3–4 weeks later a viral reactivation, particularly of human herpes virus 6 (HHV 6), cytomegalovirus (CMV) or other viruses.

There is a typical DRESS syndrome if all of these seven criteria are met in a patient. If only 1–5 criteria are met, this is an atypical DRESS syndrome^{39,40}. Although in most cases DRESS syndrome manifest itself at the skin only as a maculopapular rash, in case of delayed diagnosis it may progress up to SJS/TEN⁴¹. In that regard, DRESS syndrome should not be interpreted as an additional disease to SJS or TEN. As a part of this syndrome, various clinical manifestations of allergic delayed type reactions may occur, but in

Severity-of-illness score for Toxic Epidermal Necrolysis (SCORTEN)

- Age > 40
- Pulse > 120/min
- Tumor or histological disease
- Blister formation > 10% on the first day
- Urea > 28 mg/dl (10 mmol/L)
- Glucose > 252 mg/dl (14 mmol/L)
- Bicarbonate < 20 mEq/L

No: 0
Yes: 1



Bastuji-Garin S et al., J Invest Derm 2000; 115: 149-153

Figure 3. SCORTEN index for Toxic Epidermal Necrolysis (TEN).

T-cell reactivity in DRESS and SJS/ TEN



Figure 4. Different T-cell reactivity in DRESS and SJS/TEN.

contrast to the classical syndromes in this case they have a different pathophysiological regulation of the immune response. The pathophysiology of the DRESS syndrome differs from the classic TEN, especially in the dominant T-lymphocyte subpopulation (Figure 4). During the TEN prevail cytotoxic T-lymphocytes, but in the early phase of the DRESS syndrome Treg cells occur, which down-regulate the immune response and therefore may explain mild skin reactions compared to TEN. This functional capability of Treg cell in DRESS syndrome disappears after 3–4 weeks⁴². This functional loss of Treg cells is associated with virus activation in DRESS syndrome, but also with the activation of autoimmune diseases, e.g. autoimmune thyroiditis, type I diabetes or scleroderma¹⁴. The occurrence of anti-plakin antibodies is observed in DRESS syndrome as a marker for potential autoimmune responses. These findings led to the analysis of glucocorticoid treatment during the acute phase of DIHS and showed that the formation of anti-plakin antibodies in the patients treated with glucocorticoids occur only in 33.3%, while in the patients who were not treated with glucocorticoids in almost all the cases. This fact provides an additional rationale for the use of glucocorticoids during DRESS syndrome¹¹.

Acute generalized exanthematous pustulosis (AGEP) is defined by its characteristic clinical manifestation with multiple sterile pustular eruptions and represents a clinically

difficult differential diagnosis for pustular psoriasis. The main difference is that in drug-induced AGEP the CD8+ T-lymphocytes determine the pathophysiology of this reaction and the causative drug can be often detected in the patch test¹⁶. The separation of the AGEP from the drug-induced psoriasis became more difficult and even questionable after the detection of mutations in the IL36RN gene in most patients with pustular psoriasis without psoriasis vulgaris, which occur in the same way in patients with AGEP and lead to uncontrolled, augmented IL36-activity^{43,67}.

Drug-induced photoallergic and phototoxic reactions

Some drugs may be activated by UV light in the skin to highly reactive metabolites, which then trigger phototoxic or after a previous sensitization photoallergic reactions. In general, these drugs absorb the light in the UVA range. Currently, the most common reactions are due to nonsteroidal anti-inflammatory drugs, fluoroquinolones and hydrochlorothiazide. However, the list of possible culprit drugs is much longer, so that this type of reactions should be considered as the differential diagnosis of skin lesions in typical clinical localization at the sun-exposed areas⁴⁴.

Photoallergic and phototoxic reactions can be detected with the photo-patch test⁴⁵. A recent example is a pirfenidone which is used for the treatment of idiopathic pulmonary fibrosis and leads in up to 44% of the treated patients to phototoxic reactions¹³. The development of powder preparations for inhalation attempts to reduce the frequency of this reaction.

Management of allergic drug reactions

The basis for any diagnosis is a careful history taking, which should clarify:

- What type of allergic drug reaction experienced the patient, because depending on each individual case the diagnostic procedure may vary essentially (Figure 5),

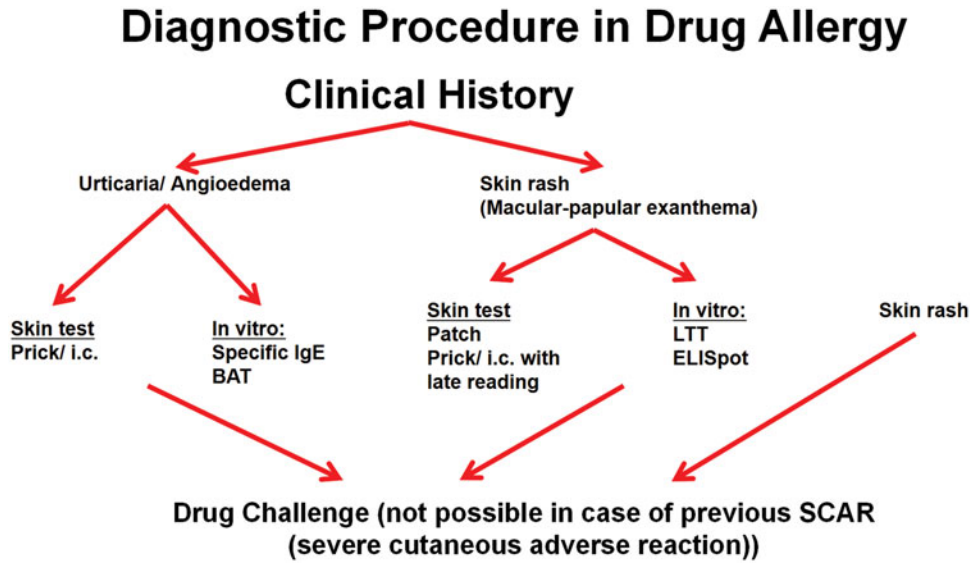


Figure 5. Diagnostic procedure in drug hypersensitivity.

- The indications for the prescription of drugs,
 - Capture of all the administered drugs,
 - The temporal relation to the duration of the drug intake and the start of allergic reaction,
 - Previous allergic reactions to small molecular substances including possible contact allergens.
- The possible risk factors for certain drugs (besides HLA associations) are presented in Table 2 and important differential diagnoses in Table 4.

Skin test

The skin is not only a signaling organ for ADRs, but also a preferred testing organ for allergy testing. In case of immediate type allergic reaction like urticaria or anaphylaxis, skin prick test and intradermal test and also *in vitro* determination of specific IgE antibodies or basophil activation test (BAT) are available. In delayed type hypersensitivity reactions, patch test is mainly used, but occasionally also skin prick test or intradermal test with delayed evaluation may be useful (Figure 5). The use of relatively high concentrations of drug in the patch test has led to an improved sensitivity of this diagnostic investigation⁴⁶. The recommended concentration of the drug in a patch test is 10%, but sometimes even 30% concentration may be useful⁴⁶.

Drug provocation test

Drug provocation tests (DPT) can be performed in immediate type allergic reactions and maculopapular drug eruptions, but not in severe, delayed occurring allergic drug reactions, especially if there is a history of a TEN. In maculopapular drug eruptions as well as in severe cutaneous allergic reactions, such as AGEP, SJS, TEN or DRESS, primarily patch test is performed, but also skin prick test and intradermal test with an early and a delayed investigation may be carried out. Furthermore, two *in vitro* diagnostic methods are available: these are the LTT or ELISPOT assay (Figure 5).

Table 4. Differential diagnosis of allergic drug reactions.

Immediate type reactions (IgE-mediated):	urticaria/angioedema/anaphylaxis
-	Carcinoid syndrome
-	Insect bites
-	Mastocytosis
-	Bronchial asthma
-	Food allergy
-	Histamine poisoning (canned fish, mackerel)
-	Latex allergy
-	Ethylene oxide/chlorhexidine allergy
-	Infections (EBV, hepatitis, parasites)
Delayed type reactions:	maculopapular rash, DRESS, AGEP, SJS, TEN
-	Acute graft-versus-host reaction
-	Kawasaki disease
-	Still disease
-	Psoriasis
-	Insect bites
-	Viral infections
-	Bacterial infections (e.g. streptococcal infections)

In vitro test

In immediate type allergic drug reactions determination of the specific IgE antibodies can be used only for few specific drugs. One reason for the limited number of assays for specific IgE-antibodies to drugs is that one needs for the establishment of these assays serum of a well-defined clinical case. In the same time, the BAT may be applied to more drugs; however, it has been evaluated for only some substances⁴⁷.

In delayed type allergic drug reactions the LTT with various modifications, such as the measured end-point – e.g. the proliferation of lymphocytes or released cytokines – may be used^{48,49}. Especially the application of the ELISPOT assay improved over the recent years. Here, lymphocytes of the patient are incubated in the plates with the culprit drug. If the patient is sensitized this leads to the activation of T-lymphocytes and to the release of cytokines which can be immediately captured by an antibody used to coat the

Table 5. Guidelines for the management of allergic and pseudo-allergic drug reactions with particular consideration of ENDA (European Network of Drug Allergy) guidelines.

Joint Task Force on Practice Parameters; American Academy of Allergy AaIACoA, Asthma and Immunology; Joint Council of Allergy, Asthma and Immunology. Drug allergy: an updated practice parameter. *Ann Allergy Asthma Immunol* 2010;105:259–273.

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membrane bottom of plates and then visualized and measured by an automated ELISPOT reader system. A number of spots found thereby correlates with the intensity of the activation of T-lymphocytes. It could be shown that this diagnostic tool, particularly in the patients with penicillin sensitization – even compared with the LTT – possesses a high specificity and sensitivity⁵⁰. A particular advantage of this test is that it is less time-consuming than the LTT and is feasible within about two days. Meanwhile, it could be shown that even with various substances, this assay provides with LTT comparable results, e.g. with drugs such as betalactam antibiotics, carbamazepine, meropenem or vancomycin¹⁶. In numerous cases, however, especially in patients with TEN the LTT provided very disappointing results. Nevertheless, investigations in which the release of granulyisin from T-cells was measured by ELISPOT assay, showed a higher significance of

Table 6. Skin test concentrations⁵².

Drug	Skin prick test	Intradermal test	Patch test
Penicilloyl-poly-L MDM	5×10^{-5} mM 2×10^{-2} mM	5×10^{-5} mM 2×10^{-2} mM	– –
Benzylpenicillin	10 000 UI	10 000 UI	5%
Amoxicillin	20 mg/ml	20 mg/ml	5%
Ampicillin	20 mg/ml	20 mg/ml	5%
Cephalosporins	2 mg/ml	2 mg/ml	5%

Table 7. Proposed concentrations for skin prick and intradermal testing for detecting a drug allergy without causing local toxic irritation^{9,72}.

Drug	Original solution (mg/ml)	Dilution (skin prick testing/ intradermal testing)
Ciprofloxacin	2	1:300 to 1:1000
Rifampicin	50	1:1000 to 1:3000
Clarithromycin	60	1:30 000 to 1:100 000

the *in vitro* assays in patients who have experienced a SJS^{50,51}. The recommendations for the diagnostic investigation of ADRs are summarized in numerous national and European guidelines (Table 5).

Antibiotics

Apart from testing for sensitization to betalactam antibiotics, skin tests with the majority of other drugs are hardly evaluated. Therefore, the risk of false negative and especially false positive reactions in the absence of control options restricts the diagnostic ability. The skin tests for investigation of anaphylactic reactions to betalactam antibiotics are well evaluated, thus there are specific recommendations for testing with appropriate substances and test concentrations (Figure 5, Tables 6 and 7).

Till recently, many of the non-betalactam antibiotics such as ciprofloxacin, clarithromycin and rifampicin had no safe and well-established concentrations for performing a skin prick test and an intradermal test. Reviewing possible irritant effects of this drugs in the intradermal test, as well as a clear evidence of an allergic reaction seen in this diagnostic procedure concentration ranges could be defined, in which no or less irritant reactions in intradermal test with ciprofloxacin, clarithromycin and rifampicin occur, but an allergic sensitization can be demonstrated (Table 7). Based on a review of current literature ENDA (European Network of Drug Allergy) elaborated for numerous drugs valid testing concentration proposals, which have been proven in clinical trials⁵². As it was shown in recent years, especially in children with a history of maculopapular rash due to betalactam antibiotics, allergic sensitization could be excluded by the skin testing and subsequent DPT. In the diagnosis of hypersensitivity to betalactam, antibiotics is also possible to determine the specific IgE antibodies. This diagnostic tool is especially useful in the medical history of severe anaphylactic reactions and may be carried out before the skin tests. An alternative is the BAT⁵³. In order to determine specific IgE against penicilloyl-derivatives most often the ImmunoCAP is used which has a rather low and variable sensitivity⁴⁷. However,

this variable sensitivity may also be the result from different populations who had been studied in particular with regard to the latency between the clinical drug reaction and the diagnostic work-up. In addition, patients with a high risk of anaphylactic reactions may be preselected by this test before *in vivo* tests will be performed. Further on the betalactam-specific/total IgE ratio in the serum may be helpful to increase the sensitivity⁵⁴.

In allergies to betalactam antibiotics the assessment of a possible cross-reaction between penicillins and cephalosporins is important. Also, a distinction between the sensitization to the betalactam ring in penicillin and the reaction to a side chain (e.g. in ampicillin or amoxicillin) needs to be done. If an allergic sensitization to penicillin can be demonstrated by the presence of IgE antibodies or by positive reactions in the skin prick test or intradermal test, an additional testing with cephalosporins will be required. Thus, in case of negative diagnostic results to cephalosporins, a DPT to a cephalosporin is recommended in order to confirm the tolerance of the patient to cephalosporins. The *in vitro* investigation for the immediate type allergic reactions may be completed by a BAT. Nevertheless, in case that both skin prick test and intradermal test are not able to confirm the allergic sensitization to penicillin and the patient's history is not significant for a severe life-threatening anaphylactic reaction, but for an anaphylaxis of grade I and II, it may be reasonable to perform a DPT with penicillin itself in order to exclude anamnestic reported allergic sensitization¹⁰.

Fluoroquinolones lead mainly to anaphylaxis and only rarely to delayed-type allergic reactions²⁶. In the recent investigation the diagnosis of allergic sensitization to fluoroquinolones was made in 69 clinical cases, from which 66 presented as the anaphylactic reaction and were confirmed by using the BAT^{55,56} or DPT^{18,56}. In only 3/69 patients, a maculopapular drug eruption was detected using the DPT^{26,57}. In addition, fluoroquinolones can be the cause for an increased photosensitivity or may also lead to a pseudoallergic reaction via binding at a mast cell specific receptor, MrgprX2, evoking release of histamine³⁰.

Perioperative anaphylaxis

The most common causes of anaphylaxis during general anesthesia are muscle relaxants. The presence of specific IgE in patients has been shown, especially in countries where pholcodine was approved as an antitussive drug. All muscle relaxants are derived from a quaternary ammonium salt and pholcodine seems to possess a structure that frequently results in IgE-dependent sensitization to such compounds. Furthermore many neuromuscular blocking drugs, including tubocurarine and atracurium, share a common mechanism with fluoroquinolones, and, as already mentioned above, may lead to a pseudoallergic reaction via binding at a mast cell specific receptor, MrgprX2, evoking release of histamine³⁰. As a part of the differential diagnosis, investigation for allergic or pseudoallergic reaction to opiates, latex, chlorhexidine, protamine, ethylene oxide and gelatin including α -Gal should be performed. Therefore, using skin tests and determining specific IgE antibodies are recommended. Also, in case of propofol, application might be helpful to check for

relevant soy allergens, since the lipophilic propofol is dissolved in soybean oil. Furthermore, propofol is frequently used together with local anesthetics because of the painfulness of propofol application alone. In addition, patients with sensitization to α -Gal cannot tolerate gelatin, so this should also be evaluated in the case of perioperative anaphylaxis.

Recent studies on the role of BAT in allergic reactions during general anesthesia have shown that it possesses a very high accuracy and specificity for muscle relaxants. Thus, in difficult cases and especially if medical history, determination of specific IgE and results of appropriate skin testing show discrepancy BAT may provide important information and lead to a definitive diagnosis⁴⁷.

Contrast media

Anaphylactic reactions on exposure to contrast media may be the result of a sensitization or pseudoallergic due to a pharmacologically mediated release of histamine. In 1000–1500 applications per year worldwide the use of contrast medium leads to a fatal incident. Thus, these group of drugs – not at least because of their frequent use – cause most of the deaths by drug allergic or pseudoallergic side effects⁵⁸. Besides anaphylaxis to the well-known iodine-containing ionic and nonionic contrast media the increasing number of allergic reactions is observed to gadolinium-containing contrast agents, which are frequently used for contrast enhancement in MRI¹⁰. In case of an allergic reaction, which can be detected by patch test or intradermal test with late investigation, the culprit chemical group should be avoided in the future. In pseudoallergic reactions, a premedication with antihistamines and glucocorticoids is recommended on the reexposure⁵⁹.

Biologics

While the symptoms of allergic reactions due to classic covalent drugs are in almost all the cases of the so-called off-target reactions, this distinction is not always possible for the biologics. Figure 6 provides a classification of ADRs to biologics, from which only a small part is represented by allergic reactions. Thus substances such as rituximab or ofatumumab are able to induce a cytokine-release syndrome after the destruction of B-lymphocytes and in this way trigger a Jarisch–Herxheimer-like reaction, which may resemble

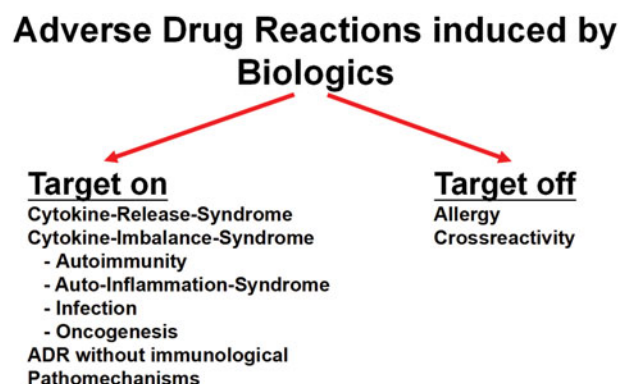


Figure 6. Adverse drug reactions (ADR) to biologics.

anaphylaxis. In case of tocilizumab (anti-IL6-receptor antibody) and similar substances, immediate type reactions as well as delayed type reactions may be possibly based on both allergic nature and a cytokine-release syndrome. In case of brentuximab (anti-CD30 antibody), anaphylaxis may occur, but its pathophysiology is still unclear⁶⁰. Furthermore, specific IgE antibodies targeting infliximab and cetuximab are able to be detected in sera of sensitized patients, but their origin is different. There is probably a direct sensitization to infliximab, while in the case of cetuximab there is likely a cross-reaction after primary sensitization to a diglycoside in muscle tissue of mammals which do not belong to the primates²⁸. Even anti-IgE antibodies can cause anaphylactic reactions, which, however, are very rare in case of omalizumab. There is a general consideration that formation of immune complexes is able to activate the complement system and therefore may lead to anaphylaxis. Omalizumab, however, binds the same region of the IgE antibodies to which the complement system also binds and that made anaphylactic reactions so rare. But this drug possesses a high specificity, which makes it very difficult to establish an animal model in order to investigate its adverse reactions, including sensitization and subsequent allergic reactions and to clarify in their pathogenesis.

A new area of adverse events is related to therapies with immune checkpoint inhibitors. They inhibit the interaction between the programmed cell death receptor 1 (PD-1) and its ligand PD-L1 and augmentation of T-cell activity by CTLA-4 antibodies such as ipilimumab⁶¹. Side effects of this therapy with compounds, such as pembrolizumab, nivolumab and ipilimumab are dose-dependent and occur in particular if there is a combination of PD-1 inhibition with ipilimumab or other ligands of CTLA-4. Cutaneous side effects include pruritus, maculopapular and erythematous reactions, but also signs and symptoms of autoimmunity such as vitiligo⁶¹. Interestingly, the application of ipilimumab to mice which lacks PD-1 after knock out of its gene resulted in new animal models for studies of allergic reactions to covalent binding drugs, because these animals develop rapidly sensitizations and hypersensitive reactions to these compounds⁹.

Declaration of interest

The authors declare that there are no conflicts of interest.

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