

Early detection of allergic diseases in otorhinolaryngology

Abstract

Asthmatic diseases have been reported since the ancient world. Hay fever for instance, was described for the first time in the late 18th century, and the term “allergy” was introduced about 100 years ago. Today the incidence of allergies is rising; almost one third of the Western population suffers from its side effects. Allergies are some of the most chronic medical complaints, which results in high health expenditures. Therefore, they have a large health and political relevance.

Caused by genetic and environmental factors, the group of IgE mediated allergies is large. It consists of e.g. atopic dermatitis, allergic asthma or allergic rhinitis. This paper aims to emphasize the ways of early diagnosis of allergic rhinitis (AR) as AR represents the most important representative of allergic diseases in ENT.

Keywords: allergic rhinitis, atopic/allergic diseases, in vitro-diagnosis, specific immunotherapy

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1 Introduction

Bronchial asthma has been reported since the ancient world, in contrary to hay fever that, was described for the first time in the late 18th century.

Interestingly, mainly aristocrats/wealthy people were those affected in former times.

Today, allergies are widespread; almost one third of the Western population suffers from its clinical implications. Allergies belong to the most frequent chronic ailment that causes high health expenditures due to high therapy costs and inability to work or go to school. In this way they have a large health and political relevance.

The word “Allergy” originates from the Greek word “allos” (“change of the original status”).

The definition is based on the concept that the exposition to an extraneous material would induce a change of reactivity in persons towards their ambience. Subsequent contacts with the substance would either increase reactivity (hypersensitivity) or decrease reactivity (hyposensitivity/immunologic tolerance). Gell and Coombs suggested classifying allergic reactions in four different immunologic mediated categories (Table 1). Type I was defined as the IgE mediated reaction that appeared immediately.

The term atopic disease is commonly used for illnesses that are accompanied by the detection of allergen specific IgE-antibodies such as atopic dermatitis, allergic rhinitis or bronchial asthma. In spite of this common property, those afore-mentioned atopic diseases show display genetic and environmental determinants and risk factors. This review paper is meant to emphasize that the early diagnosis of allergic rhinitis (AR) is the most important factor in avoiding the development of other allergic diseases namely of bronchial asthma and to achieve successful treatment.

Early diagnosis of allergic diseases is even more important considering the fact that early treatment of allergic

sensitization can prevent clinical manifestation, exacerbation or expansion of allergic diseases to other organ systems (secondary prevention).

Primary prevention on the one hand means eliminating or at least diminishing factors causing the onset of a disease. This includes alterations of causal or predisposing environmental or workplace factors. On the other hand, it means increasing the individual tolerance. Primary prevention is particularly probable for at-risk groups (genetic prestressed persons). In a certain way this might apply to the total population as well as including allergy specific health promotion.

Targets of secondary prevention are individuals that show early symptoms (e.g. bronchial or nasal hyper responsiveness or detected sensitization) and sensitized asymptomatic persons. Secondary prevention should obviate manifestation or symptom alteration.

This can be achieved by avoiding clinically relevant allergens and toxic irritant substances, consultative activities, prophylactic activities or allergenspecific immunotherapy (hyposensitization).

Avoiding new onsets of other atopic or allergic diseases can be added to secondary prevention as well.

2 Allergic rhinitis

Allergic rhinitis (AR) manifests itself in 20% of a person's lifetime, and therefore is one of the most frequent allergic diseases (Review in [1]). Its clinical manifestation often begins in early childhood, and leads to annoying symptoms that can last one's whole life. It can influence the social life, academic capacity and labor productivity of the affected patients. Typically, there is a high comorbidity described such as conjunctivitis, asthma, food allergy, atopic dermatitis, sinusitis among others. Patients suffering from allergic rhinitis seem to develop bronchial

Table 1: Classification of types of allergic reaction according to Coombs and Gell [140]

Type I	Mast cell- and IgE-mediated immune reaction (immediate reaction type)
Type II	Cytotoxic immune reaction
Type III	Immune-complex-mediated immune reaction
Type IV	Cellulär (T-cell)-mediated immune reaction (delayed reaction type)

Table 2: Classification of allergic rhinitis (according to [1])

Duration of symptoms:	
"intermittent"	"persistent"
- Less than 4 days a week	- more than 4 days a week
- Or less than 4 weeks	- and more than 4 weeks
Symptom severity:	
"low"	"moderate - severe"
- Symptoms existent	- Symptoms are existent and
- Symptoms do not affect life quality*	incriminating
	- Symptoms affect life quality*

*life quality parameter: sleeping quality, school or occupational performance, daily activity and sport activity

asthma 3.2 times more often than healthy human beings [2]. This is expressed in the initiative "Allergic Rhinitis and its Impact on Asthma (ARIA)" among others that was developed and published in collaboration with the World health organization (WHO) [1]. The comorbidities listed above motivate to diagnose and treat allergic rhinitis as early as possible (secondary prevention). It was proven that early diagnosis of allergic diseases can improve efficiency of specific immunotherapy, avoid comorbidities (e.g. bronchial asthma with patients suffering from allergic rhinitis) and can decrease the enlargement of the sensitization-spectrum [1], [3]. This considerably affects the health expenditures evoked by this disease pattern.

The social-economic consequences caused by allergic rhinitis and its comorbidities are tremendous. They arise from direct, indirect and intangible costs of the health system and the overall economy [3]. In 2000, the expenses of AR amounted to about 240 million Euros, and the expenses of the total allergic respiratory disorders (consequently including all comorbidities of AR) were estimated to be 5.1 billion Euros [4].

Therefore it must be noted that according to the white paper "Allergy in Germany", only 10% of the patients with AR are treated according to the actual guidelines, and only one third of the patients are treated at all [3].

Allergic rhinitis is clinically defined as a symptomatic disease of the nose induced by IgE mediated inflammation of the nasal mucosa after exposure to allergens. Clinically, AR can be subdivided in seasonal, perennial or work-related types, but this classification can not be used consistently. Seasonal allergens are present almost all year. Perennial allergens show seasonal upswings and

downturns in their level of exposure throughout the year. Nevertheless, allergen-exposure and symptoms do not correlate definitely, and therefore therapeutic consequences cannot be drawn directly.

A work group of the WHO suggested a new classification emphasizing the duration of symptoms (Table 2, [1]). The severity of symptoms should be defined by means of its effects on the quality of life of the patients.

2.1 Basic pathophysiological principles of allergic rhinitis

Allergic rhinitis is characterized by cellular inflammatory reactions that can be divided experimentally and partly clinically in an early phase reaction and a late phase reaction. The following processes are included in this inflammatory reaction [1]:

- Release of mediators such as histamines (essential marker of the early phase reaction), arachidonic acid meta-bolites (cysteiny leukotrienes), and kinines
- Release of pro-inflammatory and TH2- associated cytokines and chemokines
- Expression of adhesion molecules, selective cell recruiting and transendothelial migration of cells
- Activation and differentiation of eosinophiles, T-lymphocytes, B-lymphocytes, mast cells, basophiles, endothelial- and epithelial cells, fibroblasts
- Lifetime extension of inflammatory cells
- Regulation of local and systemic IgE-response, increased expression of IgE-receptors

- Interaction with the immunosystem and the bone marrow

To some extent, the release of mediators of AR, as well as symptoms of AR can be imitated by provocation with e.g. pollen allergens. This test differs noticeably from the natural allergen exposure. An early phase reaction can be reproduced in allergic patients when exposed to low doses of allergens. Late phase reactions require higher (often not physiological) amounts of allergens. The intensity of the reaction of the nasal mucosa to an allergen-exposure depends on the degree of mucosal inflammation; the intensity of the reaction varies throughout the year [5]. "Priming" describes an increased reaction caused by repeated exposure [6].

"Minimal persistent inflammation" is a new and important concept: after an allergen-exposure or after a low exposure, inflammatory parameters keep their high level even though the patients are not suffering from any symptoms [7]. The persistent allergic rhinitis is characterized by interacting allergic triggers with this sustained inflammatory reaction. The pathology is then based on a complex interaction.

An important attribute of allergic rhinitis is nasal hyperreactivity. Hyperreactivity is an intensified response to un-specific stimuli (smoke, dust, odorous substances, alterations in temperature and efforts), and leads to sneezing, nasal obstruction or nasal secretion.

Comorbidities of allergies and their early diagnosis are considerably influenced by the immunological mechanisms of the pathophysiological processes; which will be explained in detail in the following segment.

Antigen presenting cells (APC) and induction of specific sensitization

Allergens first penetrate the epithelial barrier of respiratory tract, gastrointestinal tract or dermis of the body. There, specialized and highly capable antigen presenting cells (dendrite cells, Langerhans cells) absorb allergens as total proteins and process them.

In this way, the allergen decomposes into peptides and is bound to MHC-complexes. Thus it is presented to T-cells in this kind of linkage (primarily located in the locally drained lymph nodes, where APC migrate to) [8].

Subsequently, specific native T-cells with suitable receptors are activated, they proliferate and return consistently as "Memory-T-cells" to the accordant tissue (Homing) [9]. There they can react again after allergen exposure. Besides the described specific interaction of APC and T-cells, costimulating signals are necessary to completely activate T-cells. They consist of interactions with membrane-bound molecules such as B7-group on APC or CD 28 on T-cells and interactions with soluble mediators such as interleukin 1. Lack of these signals does not lead to sensitizations, rather, it leads to anergy of T-cells (status of no excitability). This is then followed by the death of the T-cells and immunological tolerance [10]. The deactivation of immune responses matters a great deal for the regula-

tion of the immune system, especially to avoid misguided, overshooting or never-ending reactions.

2.2 TH1/TH2-Dichotomy

Besides the old classification of T-cells in CD4⁺, T-helper cells and CD8⁺ cytotoxic-suppressor cells, a new classification of T-helper cells has been established according to their cytokine production pattern. Native (not determined) CD4⁺ T-cells are called TH0-cells. They can differentiate to TH1-cells which produce interferon (IFN)-gamma, tumour necrosis factor (TNF)-alpha or interleukin (IL)-2 among others or to TH2-cells which IL-4, 5, 6 and 13 [11]. Both cell types produce their own growth factors (IL-2 and accordingly IL-4), and they inhibit the respective processing of other TH-type (IFN-gamma of TH1 inhibits TH2; IL-10 of TH2 inhibits TH1). Costimulating signals (maybe some different types of APC as well), but primarily certain cytokines can favour one course of either TH-type. So IL-4 supports the TH2-development and IL-12 favours the TH1-development [12], [13]. In turn the cytokine-ambience of mucous membranes and skin is formed primarily by the cells of epithelial cell organisations.

2.2.1 B-cells and IgE-production

High IgE-level characterize atopic diseases [14]. Mainly IL-4 (and IL-13) activates the class switch of immunoglobulins of B-cells according to specific gene products which code for various IgE- types. Furthermore, IL-4 supports the production of IgE by plasma cells [12]. The induction of class switch of immunoglobulins is also influenced by the interaction of the membrane-bound molecules CD40L of activated T-cells and CD40 of B-cells [15]. Furthermore, IL-6 can activate the immunoglobulin production generally. Moreover, several other signals exist that can manipulate IgE production. One such representative is IFN-gamma, the most important inhibitor of IgE production [16]. IFN-gamma is synthesized by TH1-cells and CD8⁺ T-cells, and therefore the cells tend to inhibit IgE production (in contrast, there are CD8⁺ T-cells that produce TH2-type cytokines too). Thus TH2-cells can initialize positive signals for IgE production (CD40L and IL-4), which can indeed can be found with atopic patients [17], [18]. The total process described above is called Allergen Specific Induction of TH2-cells, which helps B-cells produce allergen specific IgE, thus completing the induction of a type-1-sensitization.

Allergens triggering IgE mediated type-1-allergies are usually complex molecules (proteins or glycoprotein's) composed of a high number of amino acids in a defined sequence (primary structure) which is arranged in a specific way (secondary structure). In turn, bigger subunits of such a molecule fold themselves characteristically (tertiary structure). The parts of allergens recognized specifically by T-cells (variable region of the T-cell-receptor) are denominated T-cell epitopes (Figure 1); normally they are peptides (primary structure). Parts of allergens that are identified by B-cells (variable regions of the im-

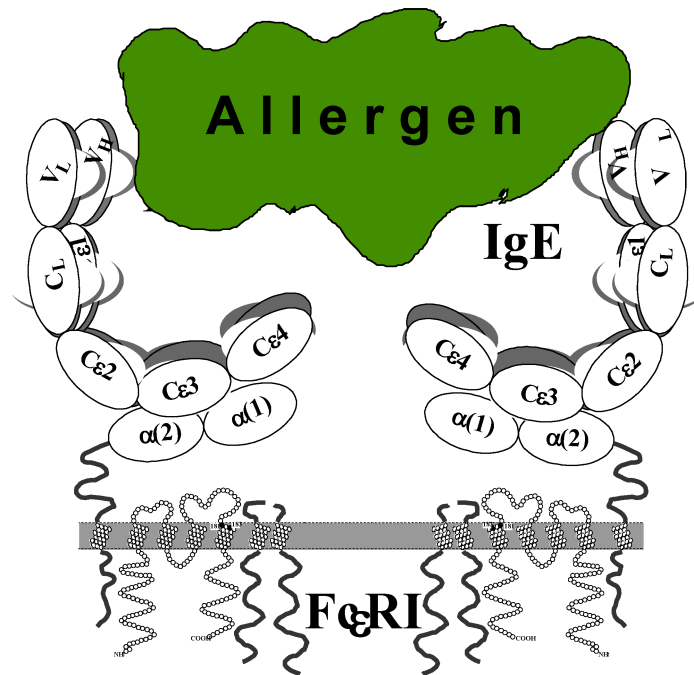


Figure 1: Interaction of allergens with mast cell-linked IgE-molecules provoke degranulation of mast cells and release of mast cell-mediators.

munoglobulins) are called B-cell epitopes (mainly dependent on conformation, tertiary structure).

2.2.2 Mast cells and “immediate phase reaction”

After occurred sensitization with allergen specific IgE-production, new allergen contact induces de-granulation of mast cells or basophiles if multiple FcεRI-bound IgE-molecules are cross-linked. Anti-IgE- or Anti-FcεRI-antibodies and non-specific factors like complement factors (C3a, C5a), thrombin as well as insect venoms, Ca-ionophore, pharmaceuticals and toxins can evoke mast cell- or basophile-degranulation, too [19]. Furthermore, “releasability” of mastcells and basophiles is modulated by cytokines such as IL-3 and neuro-transmitters like substance P (SP), vasoactive intestinal polypeptide (VIP) and somatostatine [20].

Degranulation of mast cells or basophils leads to release of several preformed mediators or newly synthesized mediators such as histamine, prostaglandins, leukotrienes, kinins, plattlet- activating factor (PAF), cytokines (IL-3, 4 and 5), proteases (tryptase, chymase, carboxypeptidase) and proteoglycans [19]. Firstly, this causes the “immediate reaction” with oedema (wheal) and erythema at the location of allergen exposure or can trigger a systemic anaphylactic reaction within a few minutes. Secondly, mast cell mediators or mediators of other cells can provoke a “late phase reaction”.

2.2.3 Eosinophiles, “late phase reaction” and chronic allergic inflammation

Usually, a “late phase reaction” (differentiate from the type of allergy that appears delayed) occurs a few hours after allergen exposure. Typically, basophiles and eosinophiles are migrating into the tissue attracted by chemotactic factors like PAF, C5a, prostaglandins and leukotrienes and cytokines [21], [22]. Those cells produce pro-inflammatory mediators and enzymes which can damage tissue like major basic protein (MBP), eosinophile-peroxidase (EPO), collagenase, phosphatase, arylsulfatase, eosinophile cationic Protein (ECP), eosinophile protein X (EPX), eosinophilie-neurotoxin (EDN), leukotrienes, prostaglandins, PAF and oxygen radicals (Figure 2) [19]. The determination of the ECP-level is a good activity-marker of atopic diseases.

At the moment, it is mainly not solved which factors could help to switch those inflammatory processes off. T-cells seem to be important in regulation of the allergic inflammation. T-cells as well as other cells like mast cells show the ability to produce important regulative mediators like IL-4. IL-4 induces e.g. adhesion molecules like VCAM-1 (vascular cellular adhesion molecule) at vessels of the affected tissue. In this way it stimulates directly migration of basophiles and eosinophiles having suitable receptors to VCAM-1, VLA-4 (very late antigen) [9]. Mediators like TNF-alpha, IL-1 and IFN-gamma play decisive roles in regulating other adhesion molecules like several selectins or integrins [23].

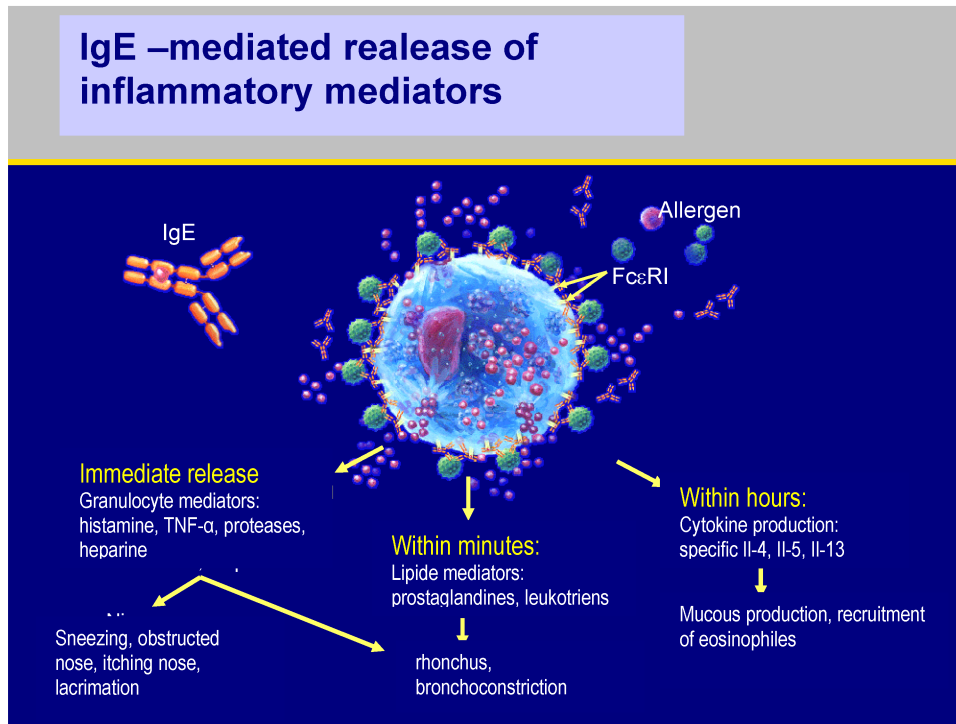


Figure 2: IgE-mediated release of inflammatory mediators

2.3 Clinical symptoms of allergic rhinitis (AR), comorbidities

The clinical symptoms of allergic rhinitis are the cardinal symptoms: sneezing, nose itching, clear nasal secretion and nasal obstruction. The rhinitis caused by pollen is characterized by sneezing, nasal secretion and concomitant conjunctivitis. In contrast, rhinitis caused by mite allergens especially generates nasal obstruction. According to the new definition, all symptoms are represented equivalently in case of the persistent rhinitis.

It was estimated [24] that about 20% of nasal allergies are caused by seasonal, 40% by perennial allergens and 40% are mixed manifestation types. First epidemiological researches according to the new definition show that about one third of all AR patients suffer from persistent AR and two thirds suffers from intermittent AR.

Comorbidities of allergic rhinitis are various and relevant. Disturbances of life quality and productivity induced by AR vary from sleep disorders to daytime fatigue [25], on to the reduction of learning aptitude of children [26]. According to a trial that included 69 children with seasonal allergic rhinitis [27], 80% suffered from pharyngitis, 70% from conjunctivitis, 40% from asthma and 37% from atopic eczema. In several trials especially asthma was discovered to be an important comorbidity of AR: children with a frequency of 32% [28] and adults with a frequency of 16% [29]. The opposite way around over 80% of asthmatic patients suffer from AR too. Sinusitis exhibits a coincidence of 25% [30]. The latter represents another important disease contributing to the patients' morbidity and should be considered differential diagnosis. This does not fit for nasal polyps that are not associated with

allergic rhinitis [31], [32]. Serous otitis media seems to be disproportionately associated with an allergy [33]. In the case of children, habitual snoring and obstructive sleep apnoea are related to allergies [34].

2.4 Increasing prevalence of allergies

Clinical trials that evaluate the prevalence of allergic diseases demonstrated decisive differences between Eastern and Western Europe. Thereby, a unique situation existed in Germany. Almost as in a natural experiment, population groups having similar genetic dispositions had been exposed to very different environmental influences in the Eastern versus in the Western part of the country over several decades. Those general living conditions have led to obvious differences in manifestation of atopic diseases in children as well as in adults. In Munich for instance, the prevalence of hay fever (8.6% versus 2.7%, $p < 0.0001$), bronchial asthma (9.3% versus 7.2%, $p < 0.05$) and bronchial hyper-reactivity (8.3% versus 5.5%, $p < 0.001$) has been reported to be significantly higher than in Leipzig and Halle in school children of the ages 9–11 years [35].

The frequency of atopic sensitizations measured by skin-prick-test showed less positive results in children from Eastern Germany than in children from Western Germany too (18.2% versus 36.7%, $p < 0.001$) [35]. In a similar comparative trial analysing children of the ages 10–12 years, it was noticed that the prevalence of atopic sensitizations in Estonia and Poland was less than in Sweden (13.6% versus 19.9% versus 30.3%, $p < 0.001$).

2.5 Natural course of atopic diseases in childhood

With increasing age the spectrum of atopic sensitizations changes in children. Primarily, during infancy IgE-antibodies are produced against food allergens especially bovine milk and egg white of chicken which can lead to different clinical symptom patterns.

They can cause dermatological syndromes (atopic dermatitis, urticaria), gastro-intestinal discomforts and rarely respiratory troubles. Sensitizations to mite, cat epithelia or other indoor allergens develop later on during toddlerhood and school age. The peak of pollen sensitization as well as the incidence of hay fever shows up around adolescence.

2.6 Risk factor for the development of allergies

In order to apply early diagnosis of allergic diseases well-directed, possible risk populations have to be defined that account for higher risks to develop allergies. In the following part risk factors for development of allergies are listed according to the actual existing data.

2.6.1 Intrinsic factors

Familiar disposition

It is well documented that a positive family history of atopic diseases represents a risk factor for developing bronchial asthma, hay fever and atopic dermatitis. The more family members are affected the higher the risk that the child will develop atopic diseases [36].

Also twin trials showed that the concordance of atopic diseases is higher with monozygotic twins than with dizygotic twins [36]. Therefore, few doubts exist that etiology of atopic diseases is mainly comprised of hereditary components. Nevertheless allergic diseases display complex syndromes with various manifestations developing from infancy to pre-school age, school age up to adulthood. It is not surprising that the heredity transmission pattern of atopic diseases does not go along with Mendel's genetics. Rather, it has to be assumed that some genes make individuals susceptible to developing asthma or other atopic diseases (polygene hereditary) and/or that different combinations of gene mutations are present in patients with atopic diseases [37].

2.6.2 Environmental factors

2.6.2.1 Type and dimension of allergen exposure

It is well proven that the development of respective sensitizations will augment with increasing concentrations of indoor allergens in childhood especially in case of genetically disposed individuals. This was illustrated convincingly in several trials for sensitizations to mite and cat epithelia. Allergen concentration of 2 µg/g dust of Der

p 1, major allergen of house-dust mite, and respectively 8 µg/g dust of Fel d 1, major allergen of cat epithelia, have been suggested as minimal doses to increase the risk of sensitization. In case of bronchial asthma significant elevated risks for developing asthma symptoms are supposed to exist when a proband is exposed to more than 10 µg/g dust of Der p 1 during infancy [37].

2.6.2.2 Nutrition

Prospective trials showed that hypoallergenic baby milk can cause a temporary protective effect on the incidence of atopic dermatitis, food allergy and infant obstructive bronchitis.

Children with genetic disposition (at least one parent suffering from allergy) developed atopic discomforts more rarely during the first one to two years of their life if they were breast feed or were fed hypoallergenic food (hydrolysates).

Other diet factors can be important in developing atopic diseases as well. A lately published Australian trial demonstrated that children with high consumption of fresh oily fish had decreased prevalences of asthma and bronchial hyper reactivity [37].

2.6.2.3 Social factors and infant infections

It has been known for a long time that allergies represent a disease of wealthy people. At present, high social status influences the development of atopic diseases such as allergic rhinitis. Furthermore, several trials displayed a highly inverse correlation between the number of brothers and sisters of a subject and the prevalence of hay fever and atopic sensitization. In various trials this coherence was documented consistently for children, adolescents and adults in many countries. Thus bronchial asthma is not associated in the same manner as hay fever and atopy [37].

This means that single children have a significantly higher risk to develop allergic sensitizations or hay fever than individuals from families with many children. In trials with a big number of cases, it has been demonstrated that older brothers and sisters have a higher protective effect than younger brothers and sisters. It was supposed that increased infantile exposure to viral and bacterial infections of the upper respiratory tract could prevent manifestation of atopic diseases [38].

This assumption is interesting regarding the different prevalence of atopic diseases in Eastern and Western European countries because the majority of children in the former DDR (low sensitization rate) went to nursery school after their first birthday. It was recently reported that very early access to nursery school before considerably reduces the risk of allergic sensitization. Furthermore, a noticeable inverse coherence was demonstrated in Lithuania and Estonia between the number of persons living in one household and atopic sensitizations: the more individuals living in the same space, the fewer people got allergies [39].

Similar coherences have been reported from other countries. Recently, an US-american research team showed that infantile non-obstructive infections of the lower respiratory tract (especially pneumonia and tracheo-bronchitis) are associated with a significant decrease of IgE-level in serum and positive skin-prick-test rate later in life of those children. Furthermore, it was demonstrated by an Italian research group that the detection of serum-antibodies against hepatitis A was associated inversely with the manifestation of asthma and atopy in early adulthood [37].

Immunological trials dealing with the IgE-regulation explain the biological plausibility of the hypothesis that infantile infections may prevent the development of atopic sensitizations and manifestations of allergic diseases. Simplified it can be assumed that two T-helper-cell-populations exist that are needed to activate anti-body producing B-cells. TH2-cells enhance IgE-production by increased release of certain cytokines like interleukin 4. By contrast TH1-cells and their cytokines (especially interferon- γ) inhibit the function of TH2-cells. The cytokine interferon- γ is also produced during the course of viral infections. It has also been demonstrated that interferon- γ is a strong inhibitor of many TH2-cell-functions and consequently inhibits the IgE-synthesis

2.6.2.4 Environmental pollution

Exposure to passive smoking

Passively inhaled smoke of tobacco contains various gases and particles with over 4,000 different chemical bonds. Tar particles, carbon monoxide, nitrogen and volatile hydrocarbon represent the most important components. Passive smoking is the main source of indoor pollution. The concentration of pollutants depends on the number of smokers, the intensity of their smoking habits, the size of the apartment and the ventilation system [40]. The pollution burden can reach immense dimensions by passive smoking since children in westernized countries stay most of the daytime indoor. Most information about the dimension and sources of passive smoking is derived from questionnaires. Some trials have measured cotinine, a degradation product of nicotine, in urine, saliva or blood as an objective parameter of pollution with tobacco smoke. But many trials showed that questionnaires coincide well with the objective measurements. Therefore it can be assumed that parents correctly report the actual consumption of cigarettes in anonymous scientific surveys [40].

Individual and socioeconomic factors are important drivers which determine the individual sensitivity to passive smoking. It can be assumed that adults reacting sensitively to the negative effects of tobacco smoke would smoke less, or would stop smoking completely. In contrast children can not choose and can not influence their parents smoking behaviour. Therefore it must be supposed that a considerable portion of very sensitive children are exposed to various pollutants of smoke.

The consequences of active- and passive-smoking-exposure have been explored in several trials. For infancy it was demonstrated convincingly that passive smoking will significantly raise the risks for developing infections of the lower respiratory tract like bronchitis and pneumonia (especially for babies and infants). Usually, the effects are higher if the mother smokes in comparison to the father. This is due to the fact that the child may be exposed more frequently through close contact to the mother or because pollutants affect a fetus.

The implications of maternal smoking during pregnancy have been investigated in 80 babies shortly after childbirth (on average 4.2 weeks later). Lung function tests were used among others. The mothers smoking habits have been documented via questionnaires and via objective parameters (cotinine-concentration in urine). Children of smoking mothers displayed significantly lower forced expiratory flows compared to healthy children.

The lung function measurements were performed shortly after childbirth and were adjusted according to multi-variant statistical models for short-time passive smoke exposure of children. So, it can be assumed that the loss of lung function test represents the implication of smoke exposure in uterus. Those damaging effects are proven until the age of 18 months. However, many factors point out that the damaging effects will last longer.

2.6.2.5 Air pollutants

Sulphur dioxide and airborne particles

It does not seem to be reasonable that high concentrations of SO₂ and airborne particles can cause the development of allergic diseases. The prevalence of asthma, bronchial hyperreactivity, allergic rhinitis and atopic sensitization has been significantly lower in regions with high concentrations of SO₂ and airborne particles like Eastern Germany and Poland than in less polluted regions in Western Germany or Sweden [40], [41].

Traffic exposure

Until now, insufficient knowledge is available about the noxious effects of exposure to traffic. It was noticed that the prevalence of non-specific respiratory symptoms and a reduction of lung function can occur in children and adults living in regions with high traffic volume [41].

In a Dutch trial, trucks caused higher effects than cars. However, at the moment, it is unknown to what extent this effect is attributed to soot or diesel exposure or to increased airborne particle exposure. So far it could not be confirmed that there are explicit effects on the prevalence of allergic rhinitis or atopic sensitization [42].

Ozone

Healthy and asthmatic subjects have been investigated regarding effects of ozone on lung function or respiratory discomfort mainly in climate trials. The extension of spirometric alterations and of symptoms like coughing, shortness of breath or inspiration-associated pain varied a lot between probands concerning a certain degree of

exposure. However those results have been reproduced in some individuals' reflecting possibly the reactivity to ozone of a particular proband [42]. It was shown by many investigators that probands adapt rapidly to a continuous exposure. Increase of bronchial reactivity to histamine and methacholine was observed in many healthy subjects after having been exposed to ozone. Therefore few indications point out that ozone might be a causal factor in the development of allergic diseases [42].

3 Diagnostic methods for early diagnosis of allergies

Diagnosis of allergic diseases is based on the typical medical history with allergic symptoms and on the results of diagnostic tests that target cell-bound IgE's in in-vivo and in-vitro tests. These diagnostic tests consist of skin tests, nasal-, conjunctival- and bronchial-provocation tests and the detection of IgE in serum. Furthermore, early diagnosis is focused normally on diagnosis in children, for which reason specific aspects of clinical investigation will be presented for patients in childhood in the following part.

3.1 Allergy diagnostics in childhood

Often first indicators for allergies can be found by questioning parents about eczema, chronic occurring gastrointestinal discomfort and frequent bronchial disorders in the first month of life. At the age of about five years, accomplishing allergy tests and their clinical benefit become as important as in adult allergic patients. The proceeding is vaguer in younger children: overall, younger children are tested more rarely by reason of faulty interpretation. Allergy testing as early diagnosis becomes more important in this period of life due to the described conclusions of immunological processes of sensitization especially in the first month and early years of life. The relevance of allergy tests is clarified in a prospective trial with children up to 2 years of age by Delacourt et al. [43]. This research group demonstrated that those children, who developed infantile bronchial asthma in all cases 18 months later, suffered from an obstructive disease and had a positive result in a prick-test against the house-dust mite *D. pteronyssinus* or to cat epithelia. The goal of optimal individual allergy diagnostics in childhood should be that children with increased risk factors for developing allergic diseases later on are identified as early as possible. Identified candidates should receive a suitable therapy with allergen avoidance, pharmacotherapy and causative allergen-specific immunotherapy.

3.2 Skin testing

3.2.1 Anatomy and physiology/immunology of skin (dermis)

The epidermis is not vascularised, and therefore the corium contains a superficial vascular plexus extending into the papillae between the two skin layers and a deep vascular plexus from where plasma and inflammatory cells can invade the skin. In addition, the corium holds nerve fibres reaching up to the epithelial layer that can contact mast cells and Langerhans-cells [44], [45]. Langerhans-cells belong to the dendrite cells and represent high potent APC [46], [47]. They are usually located in the epidermis, and migrate to local lymph nodes (SALT = Skin-associated lymphoid tissue) after having been exposed to allergens [8]. In the lymph-nodes they activate T-cells, which are normally represented in a low number in the skin. Mast cells are found in the corium and can release several different mediators. Histamine is the most important mediator in case of skin tests for type-1-allergies [48], [49]. It provokes vasodilatation (erythema) and plasma extravasations (oedema/wheel) within a few minutes.

3.2.2 Types of skin tests

Corresponding to the different allergy types, different skin tests are applied. Skin tests fit particularly well for diagnosis of type-I- and type-IV-allergies. Furthermore they are well standardized. Type-IV-allergies and contact allergies are tested by the epicutaneous test (Patch-test) [50]. In this test, allergens (mainly small haptens penetrating the epithelial barrier) wrapped in suitable vehicles are applied to the top of the unmodified skin (mostly of the back) Because the reaction is delayed, the skin is evaluated after 48 hours and 72 hours, accompanied by a generation of eczema. Skin tests for type-I-allergies are the Prick-, Scratch-, and intracutaneous test (as described below):

3.2.2.1 Skin tests for Type-I-Allergies

Pre-conditions and prearrangements

Prior to skin testing, the skin must react normally as a pre-condition. Medications and other influences should not increase or decrease skin reactivity. Allergy medication should generally be stopped (Table 3). Overshooting reactions as seen with urticaria (factitia) can be excluded e.g. by testing dermographism. Furthermore, the tested area should not include alterations like eczema, and the skin must be cleaned in order to prevent infections. The use of non-specific therapeutic agents like ointments or creams is prohibited in the testing area at the day of testing. During the period before testing, other kinds of skin irritations (physico-chemical influences) should be avoided.

Table 3: Recommended waiting period for pharmaceutical products before skin and nasal provocation testing

			Before prick testing	Before provocation testing
INN-name	Trade mark	manufacturer	Waiting period	Waiting period
Glucocorticoids – local (nose)				
Beclometason	e.g. Beconase	Glaxo	not necessary	14 days ^{1,5}
	Aquosum	Glaxo	not necessary	14 days ^{1,5}
	Sanasthmax Dosier-Aerosol	Glaxo	not necessary	14 days ^{1,5}
	Sanasthmyl Dosier-Aerosol			
Budesonid	e.g. Pulmicort	Pharma-stern	not necessary	14 days ^{1,5}
	Topinasol	Astra	max. 1 week ¹	14 days ^{1,5}
	Pulmicort Dosieraerosol	Pharma-stern	max. 1 week ¹	14 days ^{1,5}
	Pulmicort Turbohaler			
Flunisolid	e.g. Syntaris	Hoffmann-La Roche	max. 1–2 days ¹	14 days ^{1,5}
	Inhacort Dosieraerosol	Boehringer Ingelheim	max. 1–2 days ¹	14 days ^{1,5}
Momethason	Nasonex	Essex	not necessary	5 days ¹
Triamcinolon	Rhinisan	Alcon	not necessary	5 days ¹
Fluticason	Flutide	Glaxo	not necessary	14 days ^{1,5}
Glucocorticoids – systemic (In this case applies: no uncritical discontinuing!)				
Generally: long term treatment			3 weeks ³ or consider possible interactions ²	
Short term treatment >50 mg/ Prednisolon-equivalent			1 week ³ or reduction ¹	
Short term treatment <50 mg/ Prednisolon-equivalent			3 days ¹	
Generally >10 mg Prednisolon-equivalent				7 days ⁵
Antihistaminics – local				
Azelastin	Allergodil Nasenspray	Asta Medica	not necessary	24 hours ¹
Levocabastin	Livocab	Janssen	3 days ¹	3 days ¹
Antihistaminics – oral				
Azelastin	Allergodil Tabs	Asta Medica	7 days ¹	7 days ¹
Cetirizin	Zyrtec	UCB	3 days ¹ /3–10 days ²	3 days ¹
Levocetirizin	Xusal	UCB	3 days ¹ /3–10 days ²	3 days ¹
Clemastin	Tavegil	Sandoz	3 days ¹	3 days ¹
Dimetindenmaleat	Fenistil	Zyma	7 days ¹	7 days ¹
Loratadin	Lisino	Essex	2–3 days ¹ / 3–10 days ²	2–3 days ¹
Desoxyloratadin	Aerius	Essex	2–3 days ¹ / 3–10 days ²	2–3 days ¹
Mebhydrolin	Omeril	Bayer	36 hours ¹	36 hours ¹
Ebastin	Ebastel	Almirall	3 days ¹	5 days ¹

(Continued)

Table 3: Recommended waiting period for pharmaceutical products before skin and nasal provocation testing

Mast cell- stabilisators				
Cromoglicinacid (DNCG)	e.g.			
	Allergocrom	Ursapharm	not necessary ¹	24 hours ¹ /3 days ⁵
	Nasenspray	Fisons	not necessary ¹	24 hours ¹ /3 days ⁵
	Intal Aerosol	Fisons	not necessary ¹	24 hours ¹ /3 days ⁵
	Intal nasal Pulver	Fisons	not necessary ¹	24 hours ¹ /3 days ⁵
Lomupren	Mann	not necessary ²	3 days ⁵	
Vividrin Nasenspray against hay fever				
Nedocromil	Tilade Dosieraerosol	Fisons	not necessary ¹	24 hours ¹
	Irtan Nasenspray	Fisons	not necessary ¹	24 hours ¹
Ketotifen	Zaditen	Wander Pharma	>5 Tage ²	3 days ¹
β-sympathomimetics/bronchospasmolytics				
Inhalative bronchospasmolytics				generally not necessary
Fenoterol	Berotec 100/200	Boehringer Ingelheim	not necessary ¹	8 hours ¹
Orciprenalin	Alupent	Boehringer Ingelheim	8 hours ¹	8 hours ¹
Pirbuterol	Zeisin	3M Medica	not necessary ¹	6 hours ¹
Reproterol	Bronchospasmin	Asta Medica	not necessary ¹	not declared
Salbutamol	Sultanol	Glaxo	not necessary ¹	6-8 hours ¹
Terbutalin	Bricanyl	Pharma-stern	not necessary ¹	not necessary ¹
Oral bronchospasmolytics				
Clenbuterol	Spiropent	Thomae	not necessary ²	not declared
Fenoterol	Berotec	Boehringer Ingelheim	not necessary ¹	2 days ¹
Orciprenalin	Alupent	Boehringer Ingelheim	8 hours ¹	8 hours ¹
Salbutamol	Volmat	Glaxo	not necessary ¹	2 days ¹
Terbutalin	Bricanyl	Pharma-stern	not necessary ¹	not necessary ¹
Injectible bronchospasmolytics				
Reproterol	Bronchospasmin	Asta Medica	not necessary ¹	not declared
Salbutamol	Salbulair 0,5	3M Medita	not necessary ¹	not necessary ¹
Terbutalin	Bricanyl	Pharma-stern	not necessary ¹	not necessary ¹
Theophyllin	Shortly to moderately long efficient		not necessary ^{1,2}	12-24 hours ⁴
	Long efficient (retard preparations)		not necessary ^{1,2}	2 days ⁴

literature: ¹manufacturer's instructions / ²Allergy Principles und Practice, Fourth Edition, E. Middleton, Jr. et al. (eds), Mosby St. Louis, 1993/³Johannes Ring, Angewandte Allergologie, 2. Auflage, MMV Medizin Verlag München, 1991 / ⁴Allen P. Kaplan, Allergy, Churchill Livingstone New York 1985 / ⁵Arbeitskreis „Bronchiale u. nasale Provokationstests“ der DGAI, Richtlinien für die Durchführung von nasalen Provokationstests mit Allergenen bei Erkrankungen der oberen Luftwege, Allergologie 13:53-55 (1990)

Adequate treatment must be provided especially to children because patients can suffer from unexpected allergic and other reactions (presence of a physician, first aid kit and medication) [51]. Usually, children can be tested at the age of 5–6 years (for compliance reason). In this case the physician should know how to treat emergencies and should be equipped with adequate emergency materials. After testing, the person should be observed at least 30 minutes [52].

The selection of test allergens is adjusted to the individual's specific set of allergy problems. It should include the particular most frequent trigger allergens detected in taking history.

Practical aspects of skin testing

Usually, testing of type-I-allergies is carried out at the volar part of the forearms (given accessibility, reproducibility, comparability). The distance between the testing spots of two allergens must be far apart so that even two

huge reactions would not overlap one another (*Prick-test*: at least 2 cm; *intracutaneous test*: at least 5 cm). A standardized order of test allergens should be determined (test protocol as below) and reference numbers of the particular allergens should be written on the skin before testing.

The (**modified**) **Prick-Test** [53] is completed by applying a bead of allergen solution on top of the skin and pricking into the skin with a needle (or lancet) in the area of the bead [54], [55]. In this way parts of the allergen solution are inserted through the epidermal barrier into the corium by the lancet. Here the reactive mast cells are located. When using *Prick-(by-) Prick-tests* [56] the lancet is pricked into the allergen source (e.g. food) first, followed by a direct skin puncture. Non-specific toxic or infectious allergen sources should be avoided for the latter principle. A negative control (solution substance) and a positive control (diluted histamine) should always be part of Prick testing in order to see alterations from normal skin reactivity. Between the “pricking” of the various test solutions, the pricking-lancet must be cleaned thoroughly (e.g. by wiping it with a sterile swab). The Prick-test is the recommended method for skin testing [57], especially if sufficiently standardised allergen extracts are available.

With the **Scratch-test** [57], the tested area of skin must first be degreased and superficial horny lamellas must be removed by “tape-stripping”. “Tape-stripping” involves placing adhesive strips on the skin and then removing them. In order to increase sensitivity scratch lines can be created by “Prick-lancets” in the testing area additionally. This decreases the specificity of the test enormously. Afterwards the allergenic substance is wiped over the prepared area and can remain on the skin. In this case, non-specific toxic or infectious allergen sources should be avoided as allergen sources as well. As a basic principle, Prick-tests should be performed with standardized allergen extracts whereas Scratch-tests should be used only if required extracts are not available or their quality is insufficient. Therefore, the Prick-(by-) Prick-test often represents the better choice.

The most sensitive type of skin testing is the **intracutaneous test (ICT)** (1,000 times more sensitive than Prick-tests) [58], [59]. Thus they should not be performed unless Prick-testing is negative. Dangers to patients should be avoided by all means. If less potent allergen preparations are used, ICTs often represent the best choice of diagnosis. The applied testing solutions are diluted 100 times as high as those used in Prick-tests and have to be diluted further if possible. Using a tuberculinum syringe with a needle size of 26 G or 27 G 30–50 µl, the diluted testing solution is injected strictly intracutaneously (in order to avoid injections into the dermal vascular plexus). Injections of air interfere with adequate metering. A negative control and a positive control should also be carried along with this test. A new syringe and cannula must be used for every injection.

Metering of skin tests

The meter-reading of reactions from skin tests takes place after 15–20 minutes [60]. In this interval, allergic type-I reactions occur normally. It is recommended to observe the test reactions intermittently during this time period too. Late reactions can develop hours or days later, or reactions can persist (late phase reactions among others). These reactions can be allergologically relevant but normally they rarely appear at testing spots where an immediate reaction was not noticed in type-I reactions. For the metering [57], [61] the size of histamine reaction can be used in relative grades 0-III or it can be measured using absolute criteria (Table 4). Testing results should be documented in written form or in preprinted test protocols.

Table 4: Graduation of Prick-testing (intracutaneous testing)

a) related to histamine-reaction:	
∅	no reaction
(+)	light reaction
+	considerably smaller than histamine-reaction
++	smaller than histamine-reaction
+++	equal to histamine-reaction
++++	larger than histamine-reaction
b) absolute evaluation:	
∅	no wheal (<2 mm)
(+)	wheal <2 mm (2–3 mm)
+	wheal 2–3 mm (4–5 mm)
++	wheal 3–4 mm (6–10 mm)
+++	wheal 4–6 mm (11–15 mm)
++++	wheal >6 mm, pseudopodia (>15mm)

The values in brackets correspond to the wheal diameter of intracutaneous testing.

The actual used limit values should be noted on the testing protocol because the limit values for evaluation are not standardized.

3.2.3 Evaluation of testing results

Young children, old patients or certain medical conditions (e.g. poly-neuropathies, cancer-or dialysis- patients) generally have reduced skin reactivity [62]. Usually, specificity of Prick-tests is up-rated in comparison to intracutaneous tests which are more sensitive [59], [63]. Normally, positive skin tests correlate well with positive in-vitro-tests of determination of allergen specific IgE-level if the quality of allergens is good and tests are performed correctly [64].

In case of negative skin tests to a certain allergen a type-I-allergy cannot be excluded definitely if the skin reactivity is decreased (often low or no histamine related reaction) or the quality of the allergen extract is insufficient. (It might be that all relevant single allergens are not included

in the extract or that they are inactivated/degraded, expired, etc.) The evidence of non-standardized tests (e.g. Scratch-tests) has to be limited even further. Furthermore it is problematic that the skin is tested representatively for the mucosa of the upper respiratory tract in allergies of the upper air passages. When performing skin tests, only the existence of systemic sensitization can be evaluated but the clinical relevance (actuality) cannot. The medical history or further tests, especially nasal provocation tests can help. Discrepancies between skin tests and medical history should be reevaluated by taking a second medical history.

Certainly, a positive skin test does not prove that a clinically relevant sensitization exists [65], [66]. If non-specific reactions are excluded – what might be difficult in non-standardised tests (like Scratch-tests) – positive results only prove that allergen specific IgE is present on dermal mast cells, which is functionally active and could lead to mast cell de-granulation. This displays an important difference to the method of detection of allergen specific IgE-level in serum proving the explicit presence of distinct immunoglobulin classes but neglecting the functionality of IgE-molecules. In addition, allergen specific IgE persists on mast cells longer than in serum where IgEs show a half-life of a few days. Therefore IgE can only be detected in serum if it is produced during this certain period [67]. These considerations and maybe the different amount of single allergens of allergen extracts for skin tests respectively for in-vitro-testing can cause alterations between both kind of tests even though the test performance is correct.

In two constellations medical history and skin testing can predicate confidently the diagnosis of type-I-allergies: as in the case of seasonal allergens (pollen), symptoms correlate with pollen flying times; in the case of good accordance of symptoms and allergen exposure like animal contact allergies (appearance of symptoms after contact to the animal). Even though before starting therapeutic steps (e.g. initializing of allergen specific immunotherapy, extensive mite decontamination etc.) the relevance of allergens should be supported by additional provocation tests. Beside that the significance of other causes of the respective symptoms should be excluded by other medical investigations. This applies especially to perennial allergens or allergens whose exposure happens in a hidden way (e.g. mites, mould fungi). The assessment of possible type-I-allergies gets specifically difficult in the case of medical drugs (metabolites, total antigen production, few commercially available standardised test solutions), food allergies (industrial processing, digestion, arguable quality and stability of allergen extracts) and mould fungi (incomplete knowledge about its allergens, arguable quality of allergen extracts). In those events, skin test results must be interpreted carefully [68], [69].

3.3 In-vitro-methods for early diagnosis of allergies

3.3.1 Measurement of total IgE

Serum contains a considerably low concentration of antibodies belonging to immunoglobulin class E (IgE) compared to other classes of immunoglobulins. IgEs are able to provoke a specific reaction of the immune system by linking to selective, high- or low-affinitive cellular receptors [70] (Figure 3). They are structurally distinguished from IgG-antibodies by additional CH-regions at the constant region of the heavy chain. IgE-antibodies are particularly important for type-I-allergies and therefore can be used for early diagnosis of allergies.

Reference values of total-IgE for infancy have been defined in the context of multi-centre trial [71] (Table 5).

Table 5: Reference values for total-IgE

Newborn baby	<2,0 U/ml
1. year	40,0 U/ml
2. year	100,0 U/ml
3. year	150,0 U/ml
5. year	190,0 U/ml
6. year	150,0 U/ml
>16 years	120,0 U/ml

According to the applied method and epidemiological data basis normal values turned out to be a little higher or lower [72]. The maximal diffusion of the normal values was measured in the age of 6 years to 14 years [73].

3.3.1.1 Evaluation

Elevated total-IgE in the context of allergy diagnosis: The highest IgE-level could be detected in case of atopic dermatitis. Concentrations of more than 10,000 U/ml could be reached. In case of extremely high level of total-IgE (>20,000 U/ml) the differential diagnosis of cellular immune deficiency has to be excluded [74]. High total IgE combined with elevated count of eosinophiles should hint to a parasitic disease [75]. Additionally, the elevated total IgE-level can be found during the period of allergen exposure. Generally, detection of total IgE in order to screen for atopic diseases is less applicable than detection of **specific IgE** against common environmental allergens. An elevated total IgE-level does not prove the manifestation of atopic diseases; on the contrary, a normal total IgE-level does not exclude atopic diseases [76].

Atopic diagnosis in umbilical cord blood: Measurement of total IgE in umbilical cord blood was suggested in order to diagnose early risks for newborn. Such a value can be evaluated only if contamination of the cord blood by maternal blood was ruled out. Elevation of cord blood IgE >0.9 U/ml is considered as a predictive parameter for

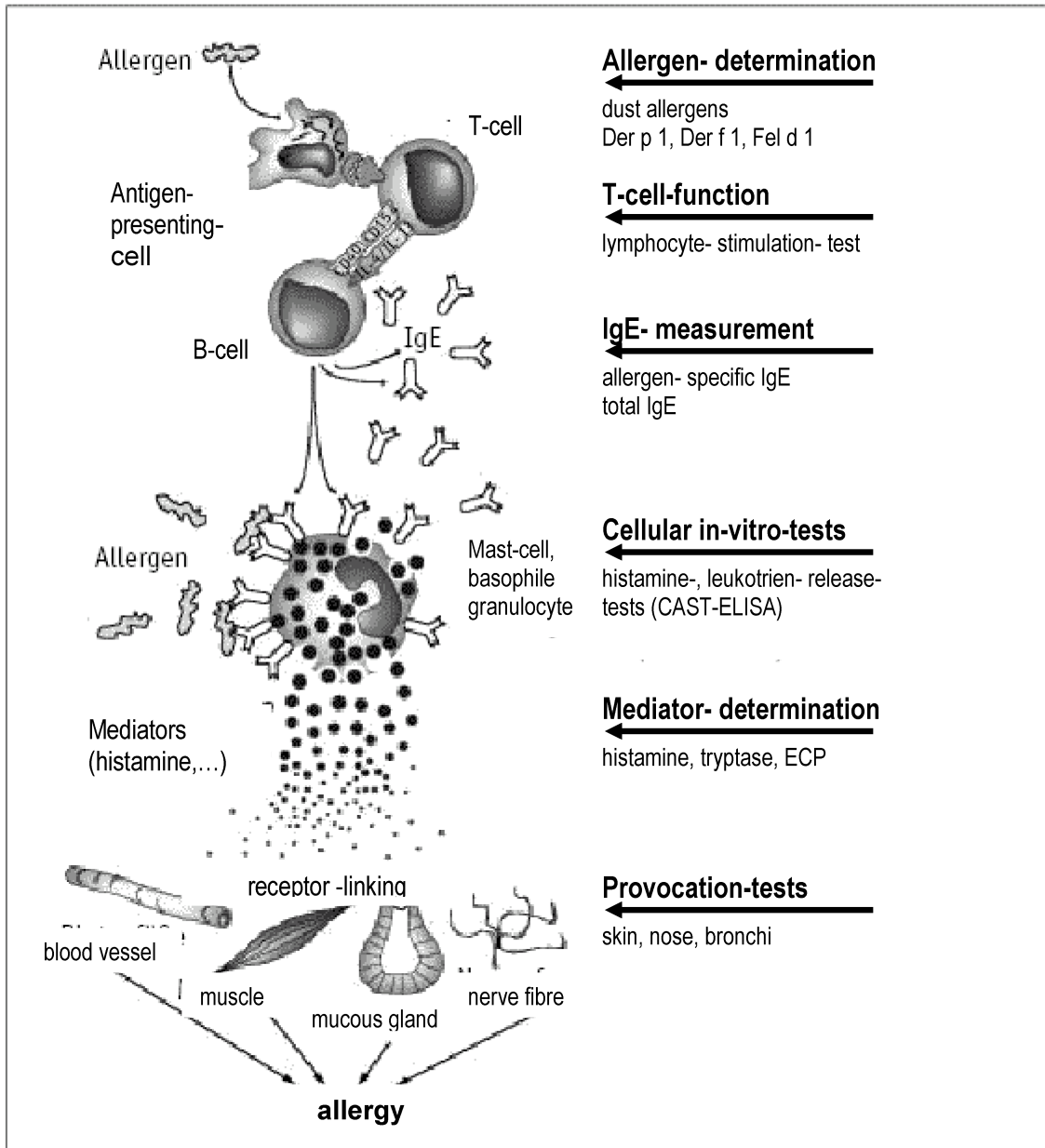


Figure 3: Model of IgE-mediated sensitization pattern and effector's phase. The arrows (right half of the picture) mark points of the IgE-reaction with different diagnostic principles and allergologic tests

the risk of atopy [77]. However values <0.9 U/ml can not exclude developing of atopic diseases. Therefore umbilical cord blood IgE-screening can not be recommended. This method should be subjected to population with risk factors like positive family history of atopy.

Immunodeficiency: A huge number of congenital immune defects, especially of the cellular system can be accompanied by elevation of total IgE [78]. In the context of immunodeficiency diagnostics measuring of total IgE-level belong to the screening procedures of the humoral immune system together with all remaining immunoglobulin groups and subgroups.

3.3.2 Measurement of specific IgE (sIgE)

sIgE represents the fraction of total IgE-antibodies in serum whose specificity to certain allergens can be de-

termined by in-vitro-tests. Detection of certain sIgEs demonstrates a specific sensitization against corresponding allergens (Figure 3). Afterwards it has to be controlled whether the sensitization is clinically relevant. Therefore sIgE represents only one parameter in the classical allergological diagnostic-concept which is also applicable for early diagnosis of allergies: medical history – skin testing – laboratory tests – provocation tests. In case of the measurement of sIgEs the quality (pattern of epitope, degree of purity) of the used allergens is important.

3.3.2.1 Indications of sIgE-measurement

Detection of sIgE in serum and skin testing are considered to be equivalent in allergy-diagnostics. In case of sIgE-

measurement in early diagnosis of allergies it can be distinguished between primary and secondary indications.

Primary indications for sIgE-analysis (before other diagnostic methods like skin tests):

- conditions, in which skin-tests are difficult to perform:
 - babies or infants
 - skin alterations of the testing area
 - Urticaria factitia
 - Other contra-indications to skin-tests or other diagnostic methods (intake of antihistamines etc.),
 - Testing of allergens that are not available for skin testing
- Danger for patients:
 - Anaphylactic shock or anaphylactic reaction in the past
 - Suspected high grade sensitization (insect venom allergy, medical drug allergy, especially beta-lactam-antibiotics)
 - intake of interfering medication like beta-blocker or ACE-inhibitor

Secondary indications for sIgE-analysis (after other diagnostic methods):

- in case of discrepancy between the results of skin test and medical history
- special selected clinical situations where additional estimation of the grade of sensitization is needed – also, as additional preparation for provocation or specific immunotherapy

3.3.3 Methods of determination of sIgE

3.3.3.1 Routine methods of determination

There exist several methods to determine sIgE being based on similar principles: specific allergen extracts and partly available recombinant allergens are connected to a solid phase or they are applied as fluid allergens to which specific immunoglobulins will link after incubation [79]. If solid phases are used, their surfaces as well as a sufficient amount and quality of allergens should assure that ideally all sIgEs can be bound. After removing unlinked immunoglobulins, radioactive Anti-IgE-antibodies marked with fluorescence or enzyme-linked Anti-IgE-antibodies are added. The linked Anti-IgE-antibodies are determined either by direct determination of radioactivity and fluorescence intensity or after adding a substance by measuring the enzymatic induced color reaction. In using a calibration curve related to known sIgE-amounts the process can be quantified. Therefore, artificially defined units or the calibration curve of total-IgE-values defined by the WHO (heterologous interpolation) are used. Hence, a real quantification of sIgEs is not possible so far. Apart from the used allergens, the testing systems differ from each other in regard to the solid phase for linking allergen and the necessary reagents as well as the following detection system (Table 6).

3.3.4 Interpretation of specific IgE-values

Screening-Tests for sIgE-antibodies: A bunch of screening tests for determination of sIgE against mixtures of food and inhalation allergens are available. They are offered especially for early diagnosis.

One difficulty of screening tests is insufficient evaluation. A positive screening test only shows that a sensitization exists against one or multiple allergens. In using semi-quantitative tests it can be assumed that negative results cannot exclude sensitizations. Generally, screening is a reasonable method if rationalization of the diagnostic method is reached. Nevertheless, the screening character of those methods should be obvious.

Table 7: Testing method for detection of allergen- and antigen-specific IgG

Qualitative procedure	<ul style="list-style-type: none"> • Ouchterlony-Technique • Immune-electrophoresis • crossbred Immune-electrophoresis
Quantitative procedure	<ul style="list-style-type: none"> • Radioimmunoassay (RIA) • Enzymimmunoassay (EIA) • Enzyme-Linked-Immunosorbent-Assay (ELISA)

In case of quantitative methods the same detection procedures can be used as used to detect sIgE. But instead anti-IgGNachweisantikörper and diluted serum sample should be used (usually 1:100). It might be difficult to interpret the quantitative antigen-specific IgG-values because standard concentrations of specific IgG-level have to be determined for every single allergen/antigen and for every used testing method. The final evaluation always has to include the clinical history and examination.

3.3.5 Allergenspecific IgG/IgG4

On the contrary to allergen specific IgE the serum-concentration of allergen specific IgG-antibodies is 100- to 1,000 times higher. Thus, less sensitive methods of detection can be used. There are several methods to determine immunoglobulins qualitatively and quantitatively (Table 7). Some companies recommend determination of IgG as a diagnostic method (especially of food allergens) on and off. Though allergen specific antibodies of isotype M, G, A can be found in sera of healthy persons as frequent as in sera of atopic individuals [80]. Production of allergen specific antibodies of this type of immunoglobulin belongs to the normal immune response to impurity exposure. Here, clinical symptoms do not correlate with the allergic type-I-reaction. Their function in the pathogenesis of bronchial asthma respectively allergy are unidentified.

Table 6: Testing methods in order to determine allergen specific IgE-antibodies (according to [141])

manufacturer, location, internet-address	name (type) of assay	allergens	solide/fluid phase	Tracing	detection system	Standard curve (units)
ADL-Matritech, Freiburg www.adl.de	CLA-Allergie-system®	div	cellulose	algE-HRP	chemi-luminescence	Calibration of internal standard curve (LU-classes)
Allergopharma, Reinbek www.allergopharma.de	Allervance® (EIA)	eig	CNBr-activated paper disk	algE-AP	photometry (405 nm)	standard curve with calibration according to WHO-IgE-standard (IU/ml)
	Allergodip® (EIA-stick test)	eig	patented support material	algE-AP	color reaction (Chromogen)	semiquantitatively (visual, classes), calibration on base of EAST (class 1–4)
Bayer Diagnostics, Fernwald www.bayerdiag.com	Magic LITE® (EIA)	eig	allergens at magnetic particles	algE-AE	chemi-luminescence	Reference with 2-point-calibration (SQ/ml) with option (kU/l)
	ADVIA Centaur Allergy® EIA)	eig	algE at solid phase and biotinilated fluid allergens	S	chemi-luminescence	standard curve with calibration according to WHO-IgE-standard (IU/ml)
BIO-RAD, München www.bio-rad.com	Allercoat 6® (EIA)	div	CNBr-activated paper disks	algE-AP	photometry (405 nm)	standard curve with calibration according to WHO-IgE-standard (IU/ml)
	Top Screen® (EIA-Streifentest)	div	nitrocellulosis	algE-PO	color reaction (Chromogen)	semiquantitatively (visual, classes)
Byk-Sangtec Diagnostica, Dietzenbach www.byk-sangtec.de	Visagnost® EIA-Streifentest)	div	nitrocellulosis	algE-AP	color reaction (Chromogen)	semiquantitatively (visual, classes)
DPC Biermann, Bad Nauheim www.dpc-biermann.de	AlaSTAT® (EIA)	div	fluid allergens	algE-HRP	photometry (405 nm)	standard curve with calibration according to WHO-IgE-standard (IU/ml)
	Immunlite 2000® (EIA)	div	fluid allergens	algE-AP	chemi-luminescence	
Dr. Fooke, Neuss www.fooke-labs.de	Fooke-RAST® Fooke-EAST® (EIA) (RIA)	eig	CNBr-activated paper disks	algE- ¹²⁵ J algE-AP	photometry (405 nm)	standard curve with calibration according to WHO-IgE-standard (IU/ml)
	Allerg-o-liq® (EIA)	eig	algE at solid phase and biotinilated fluid allergens	S	chemi-luminescence	standard curve with calibration according to WHO-IgE-standard (IU/ml)
HAL Allergie, Düsseldorf www.hal-allergie.de	ACTI.TIP® (EIA)	div	Polystyrol-pellets	algE-PO	photometry (405 nm)	Reference with 1- or 3-point calibration (AU/ml)

(Continued)

Table 6: Testing methods in order to determine allergen specific IgE-antibodies (according to [141])

Hycor Biomedical, Kassel www.hycorbiomedical.com	HYTEC® (EIA)	div	CNBr-activated paper disks	algE-AP	photometry (405 nm)	standard curve with calibration according to WHO-IgE-standard (IU/ml)
Innogenetics, Heiden www.innogenetics.de	AllergoScan® (EIA-Streifentest)	div	nitrocellulosis	algE-B, SAP	color reaction, scanner-analysis	reference curve with conjugated bovine albumine (kU/l)
Milenia Biotech, Bad Nauheim www.milenia-biotec.de	PolyCheck® System EIA-Streifentest)	div	nitrocellulosis	algE-B, SAP	color reaction, scanner-analysis	reference curve with conjugated bovine albumine (kU/l)
Pharmacia Diagnostics, Freiburg www.diagnostics.com	CAP-System® (RIA, FEIA) UniCAP® (FEIA)	eig	CNBr-activated cellulosis (capsule)	algE- ¹²⁵ J, algE-betaG algE-betaG	Gamma-Counter, Fluophotometer	standard curve with calibration according to WHO-IgE-standard (IU/ml)
R-Biopharm, Darmstadt www.r-biopharm.com	Ridascreen® (EAST)	div	CNBr-activated paper disks	algE-AP	photometry (405 nm)	standard curve with calibration according to WHO-IgE-standard (IU/ml)
	Rida Allergy Screen® (EIA-Streifentest)	div	nitrocellulosis	algE-B, SAP	color reaction (Chromogen)	semiquantitatively (visual, classes)
Intex, Weil a. Rhein www.intexpharma.de	Igevac® (EIA-Streifentest)	div	nitrocellulosis	algE-PO	color reaction (Chromogen)	semiquantitatively (visual, classes)

AE = Acridinium-Ester; AP = alkaline phosphatase; algE = Anti-IgE-antibodies; B = Biotin; CNBr = Cyanobromid; G = -Galaktosidase; div = diverse manufacturer; eig = own production; HRP = (Horseradish Peroxidase); IgE = total-serum-IgE; PO = Peroxidase; S = Streptavidin; SAP = Streptavidin-alkaline Phosphatase

Concerning their clinical relevance the importance of those antibodies is totally insecure [81]. Therefore, detection of allergen specific IgG- and IgG4- antibodies as a method of early diagnosis of allergic type-I-diseases has to be lowered.

3.4 Test systems with mast cells, basophile and eosinophile granulocytes, T-lymphocytes and their products

3.4.1 Mediators of basophile granulocytes and mast cells

Mast cells and basophile granulocytes are equipped with high-affine IgE-receptors and have the ability to release rapid inflammatory mediators and immune-regulatory cytokines [82]. They play a decisive role in the allergic type-I-reaction. Assays determining cell-specific mediators of body fluids (e.g. mast cell- tryptase, histamine) can indicate acute or currently occurred activations of those

inflammatory cells (e.g. after anaphylactic reactions). They can help to detect allergic diseases early, especially if general reactions have occurred that cannot definitely be allocated to allergic genesis.

Histamines and their metabolites can be determined in body fluids by various sensitive and specific methods; mainly using the immunoassay technique [83]. Detection in plasma or urine is not important for diagnosis of allergic diseases because of the rapid metabolism in organisms and influence of several variables. In contrast to histamine, the tryptase is degraded more slowly (serum-half-life: 2 hours), and tryptase can help to detect events of mast cell involvement retrospectively [84]. This is important in the context of early diagnosis of allergic diseases because those markers can predict severe courses of allergic reactions (anaphylaxis).

Indications for tryptase measurement:

- Questionable reactions with mast cell involvement within the last 24 hours, e.g. IgE mediated reactions by immediate type-I-allergens (insect venom, food allergy among others), anaphylactic reactions (pharma-

ceuticals, substances for invasive diagnostics among others),

- To find the cause/activator of a shock reactions (<24 h)
- Questionable mastocytosis (e.g. elevated basal tryptase-level as a hint to mastocytosis [85])
- Different variables influence the result and complicate interpretation:
 - Degree of severity of reaction
 - Time course of events and moment of blood withdrawal [86],
 - Individual aberration of mast cell activation and tryptase-level
 - No availability of common valid standard values

While high tryptase-level (>12.5 µg/l) along with low basal level can argue for involvement of mast cells, values in normal range do not mean a lot (possibly false negative). Tryptase-level measurement does not show advantages over clinical evaluation for monitoring of moderate allergic events (e.g. beginning IgE-mediated food allergy reaction after oral provocation) by reason of insufficient sensitivity [87].

3.4.2 Mediators from eosinophile granulocytes

Eosinophile granulocytes participate in the patho-genesis of allergic diseases decisively. Augmented numbers of eosinophiles can be detected in the mucous membranes of the upper and lower respiratory tract of allergic patients, as well as in the skin of patients with atopic dermatitis. The measurable mediators in serum reflect the number of eosinophiles and the degree of activation. Therefore, they demonstrate the degree and actual status of inflammatory reaction.

Several investigations are based on the detection of "Eosinophil Cationic Protein" (ECP) [88] depending on pre-analytics in a high degree: the release of ECP from eosinophile granulocytes is induced depending on the degree of activation during clotting of the whole blood (pre-analytical preparation: 1 h at room temperature, meaning 18–22 °C). ECP can be measured in other biological fluids (e.g. serum, "lavage"-fluid) by means of immunoassays (serum reference range for adults: <15 µg/l). ECP-measurement is not suited for individual prediction because of its tremendous inter-individual dispersion. Considering this fact, elevated ECP-level cannot help in diagnostics nor in an explicit allocation to a specific illness [89]. In selected cases, ECP-measurement can be used for follow-up of severe atopic diseases [90] provided that other follow-up parameters are not available.

3.4.3 Cellular allergen stimulation tests

Cellular in-vitro-tests for specific allergy diagnostics serve mainly as indirect detection methods of sensitization of basophile granulocytes (by reason of easy availability compared to mast cells). Some cellular in-vitro-test systems for type-I-allergy diagnostics use the principle of detecting mediators or cellular antigens appearing on the

surface of cells after successful activation. For this immunologic reaction blood leucocytes are concentrated by dextran-sedimentation and are incubated with allergens or other trigger factors. Surface marker (CD63) expressed after allergen stimulation or released mediators of basophile granulocytes (e.g. histamine, sulfido-leukotrienes) serve as indirect parameter for cellular bound sIgE (Figure 3). The cellular IgE-independent reactivity preparedness of involved leucocytes is reflected by other activators (bacterial peptide fMLP, complement component C5a) that cause a successful activation and mediator release.

In imitating major parts of the immunologic reaction chain of allergic reactions in in-vitro-/ex-vivo-experiments, it was expected for a long time that hereby allergic reactions could be diagnosed at an early stage. In this way, those testing methods could especially have been applied for early diagnosis. Bell-shaped dose-response-curves (Figure 4) result in using test systems with rising allergen concentrations. The slope of the curve correlates with the cellular sensitization, but does not have any relationship with the maximum of those curves. (Cellular reactivity represents a diagnostically irrelevant factor for signal transmission normally [91].) Allergen specific mediator-dose-response curves are extremely variable intra and inter-individually. Therefore a release-test is insufficient to detect sensitization if only one allergen concentration is tested. It must be added that basophiles of about 5–15% cell donors do not have the ability to release mediators after IgE-mediated stimulation even though cellular IgEs are present (non-responder). So, significance of cellular tests is less significant than direct IgE-measurement. They are methodically laborious; they do not apply for transports of probes; they are expensive and hard to conduct and interpret. Therefore they are not as relevant for allergological routine diagnostics and especially not for early diagnosis of allergic diseases. Adequate indications might be samples with low total-IgE and ineffective detection of sIgE even though a sensitization was suspected or in case of rare allergens. Due to the technical requirements and the complex interpretation they should be performed only by physicians that do have extensive experience with specialized cellular allergy tests.

3.4.4 Activation and histamine-release of basophile granulocytes

In **basophile-de-granulation-tests** cells are stained and counted before and after stimulation with allergens. At the moment, this method is not important by reason of an unacceptable dispersion. The **histamine-release-test** with allergens measures the released histamine of basophiles fluorimetrically or by means of immunoassays. As a positive control, cells are incubated with anti-IgE-antibodies [92]. The released histamine is alluded to in percentages to the total histamine of cells determined by lyses in a parallel preparation (Figure 4). This test is performed only by a few centres and is preserved mainly for special diagnostic or scientific questions because no

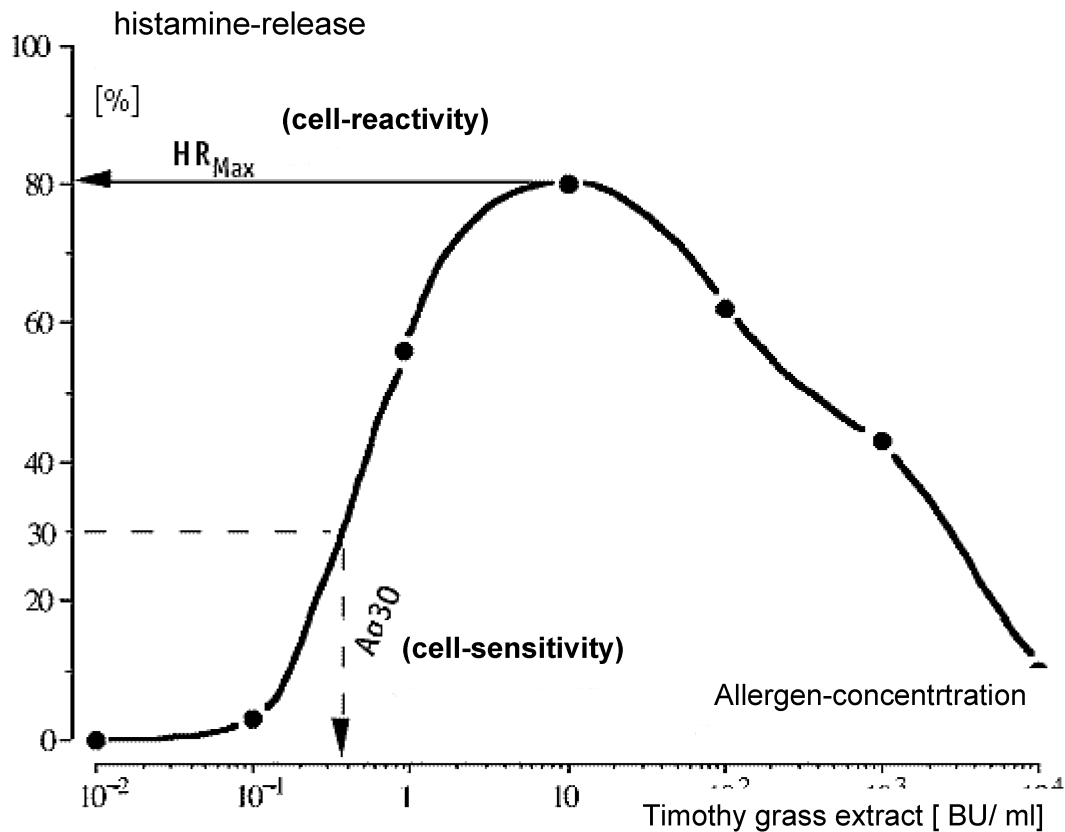


Figure 4: Histamine-dose-efficacy-curve after allergen-stimulation of human basophile granulocytes in vitro. The maximum of histamine release (displayed in percentage of total histamine-content (100%)) behaves independently of the cell sensitivity (indicated as allergen-concentration e.g. for 30% histamine-release (Ag30)). The analysis is aggravated by the individual variability (differences of multiple decimal power).

advantages exist for this test in routine diagnostics of IgE-mediated allergies compared to the method of direct IgE-measurement. Flow cytometry represents another method to investigate basophile-activation. Those methods use the new expression of CD63 at the surface of basophile granulocytes after successful stimulation via allergens. This kind of testing can be an alternative to the in-vitro-histamine-release-test in case of existent technical equipment and sufficient experience.

3.4.5 Measurement of other effectors-cell-mediators (Leucotrien-release-test)

Though other preformed or new synthesised mediators of effector-cells can be measured too especially sulfido-leukotrienes (SLT) have been used beside histamine for allergy diagnostic so far. In using type-I-allergens the leukotriene-release-test (e.g. Cellular Antigen Stimulation Test, CAST [93]) permits an indirect detection of cell-bound IgE as well (Figure 3). After mixed leucocytes have been pre-activated with interleukin-3 and have been stimulated new-synthesised SLT can be measured quantitatively in immunoassays on ELISA-basis. Basically, the same conditions count as for the histamine-release-test [94]. However the concentration of mediators will be higher due to the pre-treatment with interleukin-3 especially in donors with fewer release [95]. As a positive control for allergen induced release serves a monoclonal

anti-Fc-IgE-receptor-antibody. The SLT-release is increased in adding further stimuli like C5a, fMLP or PAF. Whether the test can deliver diagnostic information in case of intolerance-reactions by this modification [96] Whether the test can possibly deliver diagnostic information in case of intolerance reactions (e.g. acetylsalicylate acid as model for a non-IgE-mediated intolerance) by this modification [96] has to be investigated in further controlled trials.

Selected allergological diagnostic problems can indicate the use of leukotriene-release-test in particular cases [97], [98], as long as other methods (skin test, sIgE-measurement) have already been performed (Table 8). The leukotriene-release-test cannot be recommended at the moment in order to identify pseudo-allergens.

In order to evaluate the test results negative controls (stimulation with puffer and interleukin-3) and positive controls (stimulation with anti-Fcε-RI, calcium-ionophor) are supplied for IgE-mediated release of the mediator. Beside that other controls (C5a, fMLP, PAF) are attached in order to investigate other stimulation mechanisms. A positive reaction to an allergen (meaning the indirect proof of sensitization) is only indicative for a clinically relevant allergy in combination with a corresponding medical history.

The leukotriene-release-test represents a complex cellular test-system which is especially important in continuative in-vitro diagnostic and not in the routine allergy diagnostic.

Table 8: Indications for the use of leucotrien-release-tests (after skin-tests and sIgE-determination)

Medical drug allergy	In case an allergic reaction Typ I is suspected (e.g. anaphylactic shock, urticaria, bronchial asthma)
Insect venom allergy	Aberrances between medical history, skin-test and IgE-determination [97] (e. g. patients with low total-IgE <10 kU/l and specific IgE-Spiegel below detection limit)
Food allergy	At the moment this test is not important for food allergy diagnostic because there do not exist extensive studies for in-vitro-diagnostics so far.
Inhalation allergy	By reason of existent diagnostic methods leucotrien-release-test is not indicated for inhalation-allergies.

How far the test can be applied in pseudo-allergic reactions or for therapy-monitoring has to be tested in additional controlled trials. In case of explicit indications, controlled accomplishment and critical interpretation the leukotriene-release-test might be part of a specialized in-vitro allergy diagnostic potentially.

3.4.6 Determination of basophile-activation by means of flow cytometry

The activation of basophile granulocytes can be also be quantified by cytometry detection of the surface marker CD63 [99] after allergen stimulation. Single investigations with pollen- [100], insect venom- [101] and food allergens [102] proved efficiency of CD63-detection for diagnostic questions in small patient collectives. How far basophile-activation-tests by means of flow cytometry represent a potential method for early diagnosis of allergies has to be approved by further controlled trials.

3.4.7 Lymphocyte-stimulation-tests (LST)

The application of in-vitro-tests is limited in diagnostics of cellular mediated allergic reactions so far. At the moment, the lymphocyte-transformation-test is the most common test. In the LST, lymphocyte-cultures are exposed to the suspected allergen in attendance of antigen-presenting cells. The insertion of tritium-marked thymidin (3H-thymidin) is mainly used to estimate the lymphocyte-transformation. The LST proves a specific T-cell-response of mononuclear cells isolated from blood against the antigen/allergen. One problem with the LST is that it cannot be distinguished between a physiological response to the antigen and an allergic T-cell-response.

Skin tests and in-vitro-tests do not show useful results for skin lesions like exanthema after intake of medication. Therefore, drug related exanthema display the most frequent indications for LST in order to close the "diagnostic gap". Additionally, other reactions are linked to medical drugs like allergic vasculitis, aplastic anemia or hepatitis in which LST was used a diagnostic tool [103], [104]. Extremely good results are achieved by the use of LST in penicillin allergies. This led to further investigations of mechanisms of cellular immune reactions against penicillin [105], [106], [107]. Also in the case of carbamazepin and phenytoin, it was published that LST-results are meaningful when considering an immunologic setting [108]. There are some notifications of a small number of cases about positive LST in further pharmaceuticals like

nitrofurantoin, chinidin, nystatin, captopril, ibuprofen, aminophenazon oder propyphenazon [109]. Nyfelder and Pichler declared a diagnostic sensitivity of 74–78% and a specificity of 85% in one of their publications indicating that this test is not worse than other allergological testing procedures [110]. There are also reports that positive LST of blood lymphocytes in protein induced allergies like food allergies [111], [112], [113] or aggravations of atopic dermatitis are caused by inhalation allergens [114]. In order to clarify type-I-reactions and allergic contact dermatitis, more sensitive testing procedures (skin tests, specific IgE) are usually available. How easily LST can be interfered by certain substances can be demonstrated using the example of endotoxins. They can be found frequently in protein fractions and can cause false positive results of milk-protein-measurements in control individuals [112], [113]. Due to those findings, a detoxification of commercial protein fractions or allergen extracts [115] must be used before its use in LST.

Another group of allergens are classical contact allergens like nickel sulphate, chromate sals or isothiazoline [116], [117], [118]. Furthermore, LST is a suitable test to prove sensitizations against beryllium. Diagnosis of berylliosis represents the best established indication for LST [119]. In higher concentrations, some contact allergens can act as mitogenes (obligate stimuli) postulating an individual balanced titration. Whether the partly bad specificity of LST can originate from non-optimal conditions in analysis of metal compound [120] cannot be said at the moment. Good correlations can be achieved between LST and epicutaneous testing for nickel sulphate [117], [118]. Consistently, false negative reactions of LST have been observed which limit the application and acceptance of this test in routine diagnosis [106], [121], [122]. There are various reasons for negative results:

- The observed reaction in patients is not caused by immunological mechanisms.
- It was mistimed, e.g. too early after treatment with steroids, or the period has been too long between allergen contact and taking the blood sample
- A metabolite instead of the medication that was not tested in-vitro causes the allergic reaction [104], [123].
- The medical drug inhibits the insertion of thymidin by its own pharmacological effect [121].
- By adding enzymes to the test, sera 3H-thymidin is degraded enzymatically in a more extensive way than in controls [124].

- The test is not sensitive enough by reason of too low numbers of sensitized memory-cells in blood that probably react in LST.

3.4.8 Variations and possibilities to develop cellular in-vitro-test-systems

The explanatory power of LST has been improved in pre-incubating antigens with liver microsomes containing cytochrome-P450 especially in case of sensitizations against pyrazolones and anticonvulsives because metabolites can be detected as well [104], [121], [123]. New read-out-systems provide interesting perspectives for advancement of cellular in-vitro-diagnostics of allergic reactions like the measurement of cytokines (MTT-assay) or the flow cytometry display of dividing cells after adding a stimulus [125]. Flow cytometry analysis of LST with activation markers (meaning membrane molecules that can be detected more or less after antigen-triggering) seem to be less sensitive than the determination of thymidin-insertion [126], [127]. Even though flow cytometry promises to reach a higher specificity of LST in separating lymphocyte-subpopulations by functional associated marker (e.g. measurement of allergen-triggered CLA+-blood-lymphocytes in cutaneous inflammations [128], [129] or of alpha4beta7+-lymphocytes in food allergies [130]).

3.5 Therapeutic consequences from early diagnosis of allergies

The early diagnosis of allergic diseases allows a specific intervention while considering the following therapeutic aspects:

- Abatement/Removal of the patients symptoms
- Prevention of secondary damages/ development of persistent inflammatory processes in mucous membranes of the respiratory tract
- Reduction of the risk to develop a bronchial asthma and further allergy-associated diseases
- Reduction of the risk to develop further sensitizations

The therapeutic efficiency of allergen specific immunotherapy with subcutaneous injections (SCIT) with allergens and respectively chemically modified allergen extracts (Allergoids) could be demonstrated in several trials (reviews in [131], [132], [133]).

SCIT is also a cost-effective treatment in consideration of economic factors like the reduction of the medication usage, increase of productivity and respectively decrease of absences of work and diminished prevalence of new sensitizations and allergic asthma [134], [135].

3.6 Preventive effects of SIT with regard to the development of allergic bronchial asthma

The data of the PAT-trial is important in order to evaluate the early treatment by SIT. This European multi-centre trial showed a significant decrease of the development of a manifest bronchial asthma (Figure 5) [136].

They have treated 144 children with allergic rhinitis (AR) either with SCIT or medicinally for a period of three years. Interestingly, it was observed that children showed a significantly decreased prevalence of asthma having been assigned to the SIT treated group after three years of therapy. Furthermore, a preventative long-term effect could be proven regarding the prevention of asthma. Even seven years after finishing SCIT, children developed significantly fewer allergic asthma than the control group [137]. Considering these results, the immunotherapy is the only known therapy that reduces the development of bronchial asthma in patients with allergic rhinitis.

3.7 Prevention of additional allergic sensitizations

Explicit evidence exists that the prevalence of new allergic sensitizations can be decreased by SCIT. A prospective trial was published with 44 children suffering from house dust mite allergy. They showed that the rate of new sensitizations can be reduced significantly by a 3-year SCIT [138]. They also discovered similar preventive effects in a retrospective open-label-trial with 8,396 patients for a observation period of seven years [139].

4 Summary and future prospects

Allergic diseases belong to the most frequent diseases of humanity. Their prevalence is growing dramatically in all industrialised countries.

Allergies often cause atopic dermatitis, allergic rhinitis or bronchial asthma in early childhood. Besides considerably restricting symptoms, allergies affect the life quality of patients, their social life, and school and work productivity.

Allergic diseases are characterized by various comorbidities which arise depending on the duration of illness. Additionally, the affected tissue can be damaged. Furthermore, a chronic persistent inflammatory alteration of tissue can occur which may be irreversible. During the course of the disease, the number of allergens to which the individual patient reacts can also increase. All these issues combined can have tremendous consequences on the direct, indirect and intangible costs of a health system and overall economy. Diagnosis and treatment of allergic diseases as early as possible can prevent some of the outcomes (secondary prevention).

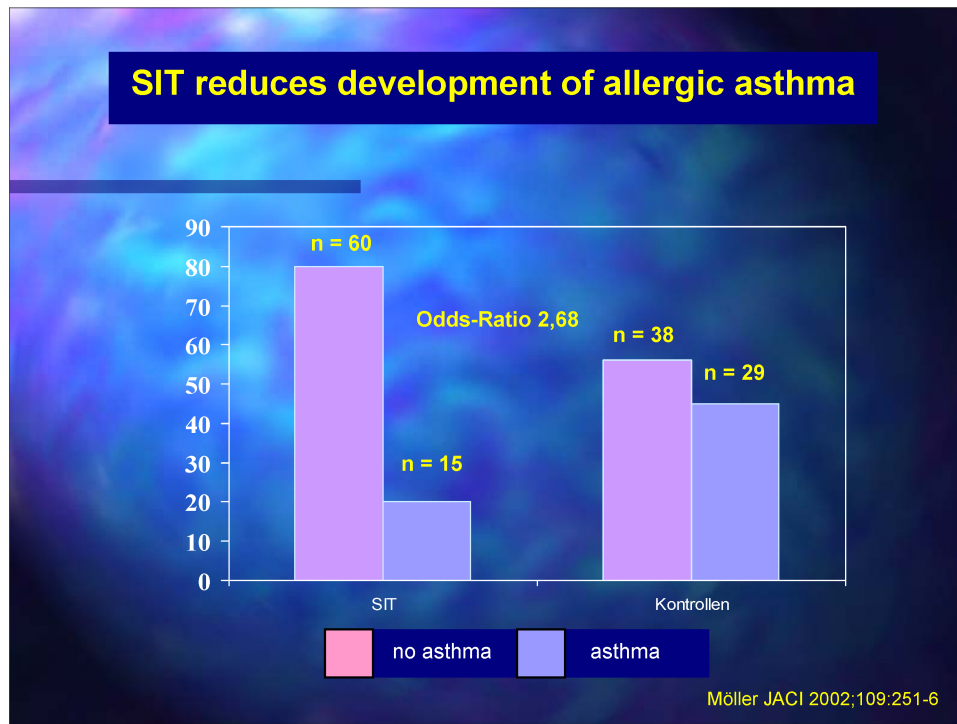


Figure 5: Allergen specific immunotherapy can avoid the development of allergic bronchial asthma in patients with allergic rhinitis (according to [136])

It was demonstrated that early diagnosis of allergic diseases makes specific immunotherapy more efficient. In this way comorbidities can be avoided (e.g. bronchial asthma in patients with allergic rhinitis) and the expansion of sensitization can be limited. In the future, improved diagnostic methods can further enhance accurate detection.

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