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The prevalence of asthma and allergy increases: a world-wide problem

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Samenvatting

Dit rapport geeft een overzicht van de actuele prevalentie van allergische aandoeningen en laat zien dat allergie een belangrijke risicofactor is van astma. Overgevoeligheid voor allergenen, in het bijzonder huisstofmijt, is geassocieerd met een verhoogd risico voor de latere ontwikkeling van astma. Daarnaast geeft het rapport een overzicht van de verschillende determinanten van atopische ziektebeelden, zoals genetische, omgevings- en leefstijl factoren. Tot slot wordt een aantal biomarkers gepresenteerd, die gebruikt kunnen worden de incidentie van reeds ontwikkelde atopische ziektes verder te onderzoeken in epidemiologische studies. De afgelopen 20 tot 30 jaar is de prevalentie van astma met ongeveer 50% per tien jaar toegenomen. In dezelfde periode nam de prevalentie van hooikoorts eveneens snel toe. De genetische achtergrond speelt een belangrijke rol bij atopie, aangezien het risico om atopisch te zijn 50% is als een van de ouders atopisch is, en 60% indien beide ouders atopisch zijn. Omgevings- en leefstijlfactoren, zoals infecties op jonge leeftijd en blootstelling aan luchtverontreiniging kunnen gedeeltelijk verantwoordelijk zijn voor de waargenomen stijging in de prevalentie van allergische aandoeningen.
In Nederland is de prevalentie en de incidentie voor astma in kinderen en volwassenen ongeveer gelijk met andere westerse Europese landen, waarbij de prevalentie van allergie voor luchtwegallergenen in kinderen tussen 7 en 12 jaar inmiddels is gestegen naar 30%.
Op omgevings- en leefstijlfactoren zijn in principe interventies te plegen. Het is echter van belang om de aanleg van een kind om overgevoelig te worden in de vroege fase van zijn of haar leven te voorspellen, omdat preventieve maatregelen minder effectief zijn nadat de sensibilisatie reeds is opgetreden. Dit illustreert duidelijk de hoge behoefte aan ‘markers’, die het hoge risico op toekomstige sensibilisatie in jonge kinderen op betrouwbare wijze kunnen voorspellen. Onlangs kwamen we naar aanleiding van resultaten uit een cross-sectionele epidemiologische studie tot de conclusie, dat een relatief hoge concentratie aan uitgeademd NO op jonge leeftijd van voorspellende waarde zou kunnen zijn voor een risico voor allergie op latere leeftijd. Deze waarneming dient te worden bevestigd in een longitudinale epidemiologische studie.

Summary

The present report reviews the actual prevalence of allergic diseases and outlines that allergy is an important risk factor for asthma. Hypersensitivity to allergens, in particular house dust mite, is associated with an increased risk for later development of asthma. In addition, the report gives an overview of the various determinants of atopic diseases, such as genetic, environmental and lifestyle factors. Finally, a number of biomarkers is presented which can be applied to further investigate in epidemiological studies the incidence of established atopic diseases. Over the past 20 to 30 years the prevalence of asthma has increased by some 50% every ten years. In the same period the prevalence of hay fever has increased rapidly. Genetics play an important role in atopy, considering that the risk to be atopic is 50% if one parent is atopic and 60% when both parents are atopic. Environmental and lifestyle factors such as childhood infections and exposure to air pollution, may be partially responsible for the observed increase in the prevalence of allergic disease.
In the Netherlands, the increase in the prevalence of asthma in children and adults seems to be similar compared to other Western European countries, by which the prevalence of allergy to aeroallergens in children aged 7 to 12 years has recently been shown to be increased to 30%.
Environmental and lifestyle factors are in principle subject to intervention, but it is important to predict in the early stage of its life that a child is prone to become hypersensitive, because preventive measures are less efficient after sensitisation has become apparent. This clearly illustrates the urgent need for markers that reliably predict a high risk for future sensitisation in young children. Recently, we concluded from a cross-sectional epidemiological study that a relatively high level of exhaled NO at young age might be a suitable predictor of an increased risk to develop allergy later in life. This observation needs confirmation in a longitudinal epidemiological study.

1. Introduction

1.1 Time trends of allergic disease

Between 100 and 150 million people around the globe suffer from asthma and this number is still rising [1]. World-wide, deaths from this condition have reached numbers of over 180,000 annually [1]. Experts are struggling to understand why the world-wide prevalence rates are, on average, rising by 50% every decade. Similarly the prevalence of hay fever increased rapidly over the past decades and is still increasing. It is estimated that roughly 20% of the population is now atopic [1].

1.2 Definitions used

Atopy is a genetically determined condition of individuals that makes them prone to develop allergy. Atopy does, however, not necessarily imply the presence of symptoms but an atopic constitution is strongly associated with allergic disease such as asthma, hay fever and eczema. In this context, allergy refers to the immediate (type I, IgE-mediated) hypersensitivity to environmental antigens. Asthma is defined as a clinical syndrome that is characterised by airway inflammation, variability of lung function and airways responsiveness. Bronchial asthma with or without allergy or atopy can be distinguished by IgE antibodies to allergens.

1.3 Markers of allergic disease

A positive skin prick test to house dust mite or increased specific IgE titres indicate the presence of type I hypersensitivity which represents an increased risk for later development of asthma. As such clinical assessment of type I hypersensitivity can predict whether a child is at risk to develop asthma. This clearly illustrates the urgent need for a reliable marker that predicts in a child an increased risk for future sensitisation.

Recently, we measured exhaled NO in various groups of school children. Our preliminary results from a cross-sectional study in 275-370 school children (aged 6-11 years) showed that 84% of the children exhaling a high concentration of NO were allergic (positive skin test or specific antibodies to allergens.

1.4 Scope of the present review

The purpose of the present paper is to describe rising trends in the prevalence of asthma and allergy and not to review epidemiology of allergic diseases in full depth. Like the other reviews covering this topic, this survey is hindered by methodological problems in defining childhood asthma and wheezing illnesses [2] which may partly explain the world-wide variation in the prevalence of childhood asthma and allergic conditions. The discussion remains open whether to use symptoms of asthma such as wheeze or shortness of breath, a doctor's diagnosis of asthma, or some combination of symptoms and airway hyper-responsiveness to estimate the prevalence of this disease. For instance, studies that use the presence of wheezing as an indicator of asthma may overestimate the prevalence of the disease because of the poor specificity of wheezing symptoms for asthma. Note that in some

languages there is no term for 'wheeze' [2]. It is evident that such differences in scientific designs troubles comparison of the many reports available on this item.

Furthermore, we discuss the T helper balance as a theoretical basis for atopy, we give information about the genetic background of atopy and review risk factors of atopic disorders.

Finally, we discuss early biomarkers of allergic disorders in children including a potential predictor of an increased risk to develop allergy later in life.

2. Prevalence of asthma*

Marked regional variation in the prevalence of asthma exists. For example a relatively high prevalence is observed in English-speaking countries. In U.S. the prevalence increased from 3.1% in 1980 to 5.4% in 1994. Combined prevalence of diagnosed and undiagnosed asthma among inner city children were 26-27% at 9 to 12 years of age in Detroit and San Diego. Annual Health Interview Surveys have disclosed increases in self-reported prevalence of asthma in the United States from 30.7 per 1,000 in 1980 to 53.8 in 1993-94 [3;4]. Rates have been higher for blacks than whites, increasing from 34.0 per 1,000 in 1980 for blacks to 57.8 in 1993-1994, compared with increases for whites from 30.4 to 50.8. Increases across time have occurred in all age groups, but the greatest proportionate increase has occurred in children less than 5 years of age with a 160% increase from 22.0 per 1,000 in 1980 to 57.8 in 1993/1994 [3]. For children 5 to 14 years of age, prevalence increased from 42.8 per 1,000 in 1980 to 74.4 in 1993/1994, an increase of 74%. Increases in prevalence occurred in all four regions of the country.

According to the UCB Institute of Allergy in Belgium, which recently reviewed the prevalence of allergic disease, asthma has doubled in ten years in Western Europe as a whole [1]. Around 8% of the Swiss population suffers from asthma now as against only 2% some 25-30 years ago [1]. In Germany, there are an estimated 4 million asthmatics, and in The Netherlands the estimated number of asthmatics is 1.6 million [1]. In the Netherlands, recent figures on the prevalence and incidence of asthma can be derived from three studies. One study from 1994 used doctor diagnosed asthma while the other two studies (from 1992 and 1997) scored asthma symptoms in an epidemiological population-based study via questionnaires. Based on doctor diagnosed asthma the prevalence and incidence was 1.1% and 0.4%, respectively, while the prevalence for asthma as assessed by questionaires was about 15% [5].

In English-speaking countries the incidence of allergic diseases appears to be much higher. For example in the U.K. the prevalence of diagnosed asthma and symptoms strongly suggestive of asthma in children has increased during the last decades at a rate of 5% a year [6]. In England again, Burr and colleagues [7] repeated exercise provocation tests in schoolchildren in 1973 and 1988. They reported a significant increase in the prevalence of wheeze and history of asthma as well as a doubling in the prevalence of bronchial hyper-responsiveness over this time period. Overall, the results of the exercise provocation tests suggested that both mild and severe asthma had become more common. Increases of a similar magnitude have been observed in Sweden, Australia, Switzerland, Norway, U.S., New Zealand and Taipei [6]. In Japan there are about 3 million asthmatics of whom 7% have severe and 30% have moderate asthma and in Australia, 16% of the children under the age of 16 is affected [1]. The surveys by Peat and colleagues in Australia [8] showed that, besides a doubling in the prevalence of wheeze in the past 12 months, airway hyper-responsiveness increased 1.4 to 2-fold in 8-10-year-old schoolchildren over the years 1982-92.

* The paragraphs on prevalences and atopy as risk factor are mainly based on reviews by Prof. von Mutius, The rising trends in asthma and allergic disease, Clin. Exp. Allergy 28, suppl. 5, 45-49, 1998 and Dr. Sly, Changing prevalence of allergic rhinitis and asthma, Ann. Allergy Asthma Immunol. 82, 233-252, 1999.

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In developing countries the incidence of the disease varies greatly. India has an estimated 15-20 million asthmatics (rough estimates indicate a prevalence of between 10% and 15% in 5-11 year old children); in the Western Pacific Region of WHO, the incidence varies from over 50% among children in the Caroline Islands to virtually zero in Papua New Guinea. In Brazil, Costa Rica, Panama, Peru and Uruguay, prevalence of asthma symptoms in children varies from 20% to 30%. In Kenya, it approaches 20% [2]. Van Niekerk and co-workers [9] assessed bronchial responsiveness to exercise challenge tests in 6-9-year-old black children in an urban and rural area in South Africa. In the urban population the prevalence of airway hyper-responsiveness was 3.2%, whereas among 671 children in rural Transkei only one subject had a significant drop in FEV_1 after exercise challenge.

It is concluded that, despite the use of different definitions of the disease and different study designs, most studies conclude that the prevalence of childhood asthma has rapidly increased during the last decades.

3. Prevalence of allergy*

Most of the surveys that have shown increased prevalence of asthma have also shown increased prevalence of other allergic diseases, such as hay fever and eczema. In the U.K. three birth cohorts in 1946, 1958, and 1970 have shown a marked increase in the prevalence of eczema (5.1, 7.3, and 12.2%, respectively) [6].

An increase in the prevalence of hay fever (measured by skin test or specific IgE) and atopic eczema has been reported in England and Scotland [4;10], Australia [8], New Zealand [11] and Sweden [12]. For example, Ninan and colleagues [13] in Scotland studied schoolchildren 8-13 years of age in 1964, 1989 and 1994. The prevalence of hay fever increased from 3.2% to 11.9% and 12.7% in the third survey, whereas the prevalence of atopic eczema rose even more strongly: 5.3% in 1964, 12.0% in 1989 and 17.7% in 1994. In a recent study in the Netherlands, 33% of 1144 children at a age of 7-12 yr. proved to be skin prick test positive and 29% (n=883) of these children showed increased IgE antibody titres to aeroallergens [5].

Unfortunately, very little information is available on changes over time of objective markers associated with hay fever such as atopic sensitisation [17]. Nakagomi and colleagues [14] measured specific serum IgE antibodies to a panel of 16 inhalant and food allergens in 457 schoolgirls 13-14 years of age in 1978, 1981, 1985 and 1991 in Japan. These authors demonstrated in a preliminary report a significant increase in the prevalence of atopy, i.e. one or more positive reactions from 21.4% in 1978, 25.0% in 1981, 35.5% in 1985, 39.4% in 1991. In the same cohort this group [18] later showed an increased prevalence of elevated serum IgE and IgG4 antibodies. However, Peat and colleagues [8] did not find in Australia an increase in skin test reactivity to a panel of aeroallergens, although the prevalence of asthma and airway hyper-responsiveness had increased over time.

Several recent surveys performed after the reunification of West and East Germany have documented large differences in the prevalence of hay fever, atopic sensitisation, asthma and airway hyper-responsiveness in children and adults between East and West Europe [1;15-17;19]. In Sweden and West Germany the prevalence of atopic diseases in children was significantly higher than in the eastern areas of Poland, the Baltic States and East Germany. The frequency of atopic sensitisation measured by skin prick tests in East Germany children was about half the proportion of children living in West Europe [1;19]. Accordingly, significantly fewer children living in East Germany were hyper-responsive to a cold air hyperventilation challenge than their peers in the West. Similarly, among adults living in the eastern and western part of Germany significant differences in the prevalence of asthma, airway hyper-responsiveness and atopy were found [15;16].

Since the reunification of West and East Germany life style and living conditions have changed in the eastern part of Germany. If western life style is indeed associated with an increase in the manifestation of atopic diseases then increases in the prevalence of these conditions should occur in these areas. In children who were brought up in their first 3 years of life in the German Democratic Republic (GDR) and exposed to westernised living

* The paragraphs on prevalences and atopy as risk factor are mainly based on reviews by Prof. von Mutius, The rising trends in asthma and allergic disease, Clin. Exp. Allergy 28, suppl. 5, 45-49, 1998 and Dr. Sly, Changing prevalence of allergic rhinitis and asthma, Ann. Allergy Asthma Immunol. 82, 233-252, 1999.

conditions after their third birthday when Germany was reunified, the prevalence of hay fever (2.3% vs. 5.1%) and atopic sensitisation (19.3% vs. 26.7%) significantly increased over the years 1991/92-1995/96, whereas the prevalence of asthma and airway hyper-responsiveness remained virtually unchanged [15;16].

Apparently, important differences in the development of childhood asthma versus hay fever exist. Atopic diseases develops at various ages but childhood asthma starts before the age of eight in most affected subjects, whereas the incidence of hay fever generally peaks around adolescence [20;21]. Similar developmental characteristics have been shown for the process of specific IgE antibody production, food allergies being the most common type of allergy in the first years of life and sensitisation to mites and pollen developing thereafter [22;23]. Realising that the children in the GDR were exposed to western life style and living conditions from three years of age, factors operating in the very first years of life may be particularly important for the onset of childhood asthma later in their life. Various factors have been proposed such as sib-ship size, attending kindergarten, nutritional habits, the socio-economic status and quality of housing conditions. It is therefore suggested that environmental factors occurring beyond infancy determine the expression of this illness.

In this respect it is further of interest that farmer's children which come more frequently in contact with infectious materials show a lower prevalence of allergic disease than children of non-farming families [24]. The higher prevalence of allergic diseases with urban residence rather than with rural residence during the first 2 years of life is confirmed by some [25]. Others however, observe a higher percentage of sensitisation to pollen but not to pet allergens in children living in rural area as compared to those living is an urban area [26].

4. Risk factors of atopic disorders

4.1 The Th1/Th2 balance as a theoretical basis of atopic disorders

T cells play a central role in the specific immune response as both regulators (T helper cells) as effectors cells (T cytotoxic) of the immune functions. From these, the T helper cells are considered as the most important in atopy. These T helper cells can be divided into two subpopulations, namely T helper 1 (T_h1) and T helper 2 (T_h2). A number of factors affect the differentiation of the naive T helper cell precursors into a Th1 or Th 2 cell. These include the affinity of the T helper cell receptor for allergens and the presence of cytokines. T_h1 cells are involved in cell-mediated immunity (control of invading micro-organisms), while T_h2 cells dominate parasitic infections and allergy [27]. The balance between the two subsets of T-cells (T_h1 and T_h2 cells) is pivotal for allergic sensitisation. T-helper subsets can be distinguished by their cytokine profiles. T_h1 cells produce mainly IL-2 and IFN-γ and T_h2 cells produce mainly IL-4, IL-5, IL-10, and IL-13. The cytokines IFN-γ and IL10 may also induce a shift in the balance between T_h1 and T_h2 responses [28]. Via the release of certain cytokines the subsets may mutually modulate the response. For instance, release of IFN-γ by T_h1 cells will inhibit the T_h2 response and will recruit cytotoxic lymphocytes and macrophages, release of IL-10 by T_h2 cells will inhibit the T_h1 response and initiate production of IgE and Ig2a and the recruitment of mast cells and eosinophils.

During pregnancy foetal immunity is dominated by humoral (T_h2) responsiveness [29;30] which is preserved in early infancy. Later, viral and bacterial infections adequately replace the T_h2 phenotype of the child to a T_h1 phenotype. It has been suggested that besides childhood infections, air pollution has immune-regulatory actions which may affect the T_h1/T_h2 balance. The establishment of a T_h1/T_h2 balance during early childhood and the final tuning at an age of 4-5 years [31] implies that very young children are notably vulnerable to environmental and life-style stressors affecting the T_h1/T_h2 balance. At older age subjects remain, however, sensitive to a shift in the balance by such factors. NO (nitric monoxide) also affects the T_h1/T_h2 balance by inhibiting T_h1, but not T_h2 cells [32]. As a result, NO promotes allergy.

4.2 Allergy as risk factor for asthma*

Exposure, especially in infancy, to indoor allergens (such as house dust mites, cats and cockroaches) represents a major risk factor for developing asthma, indicating allergy as an established risk factor for asthma.

A number of studies showed a highly significant relationship between prevalence of asthma and serum IgE concentration [33-35]. There was no asthma in subjects with the lowest IgE levels. Allergy prick testing of a sample of the U.S. white civilian population (4,295 subjects 6 to 24 years of age) in 1976-1980 showed significant associations between asthma and reactivity to house dust mite (HDM, Dermatophagoides *pteronyssinus*) and Alternaria [36] while allergic rhinitis was associated with reactivity to ragweed and ryegrass.

* The paragraphs on prevalences and atopy as risk factor are mainly based on reviews by Prof. von Mutius, The rising trends in asthma and allergic disease, Clin. Exp. Allergy 28, suppl. 5, 45-49, 1998 and Dr. Sly, Changing prevalence of allergic rhinitis and asthma, Ann. Allergy Asthma Immunol. 82, 233-252, 1999

Allergy skin testing with 13 inhalant allergens in 380 Australian schoolchildren in 1982, when they were 8 to 10 years of age, and again 2 and 4 years later showed increases in prevalence of at least one positive skin test from 24% to 39% [37]. Allergy at 8 to 10 years of age was a risk factor for asthma, wheezing, and bronchial hyper-responsiveness.

A prospective study of 67 British children at risk for allergy because of a parent with asthma or hay fever, identified high exposure to HDM in early childhood as a risk factor for asthma [38]. Analysis of house dust samples collected in the homes of the children when they were 1 to 2 years of age showed no child exposed to less than 2 μg HDM allergen per gram of dust had become sensitised by 11 years of age. All but one of the children with current asthma at 11 years of age had been exposed at 1 year of age to more than 10 μg house dust mite allergen per gram of dust, which conferred a relative risk of asthma of 4.8.

A studies of 3,581 children 8 to 11 years of age in Australia showed significant associations of wheezing within the previous year and hay fever with at least one positive prick test to a group of 13 aeroallergens [37]. Sensitivity to HDM had the strongest independent association with current asthma (bronchial hyper-responsiveness and wheezing within the previous year). Risk of asthma also has been associated primarily with sensitisation to HDM and cat in 13-year-old children in New Zealand [39]. Allergy prick testing of 343 children 7 to 12 years of age recruited from a general paediatric practice in U.S. identified *Alternaria tenuis* sensitisation as a risk factor for recurrent wheezing [40]. Logistic regression also identified cat allergen and *D. farinae* as risk factors.

Accordingly, allergy is a risk factor for asthma, but the specific allergens of importance depend upon intensity and probably duration of exposure. Intermittent exposure to a seasonal allergen may not elicit airway inflammation of sufficient duration to cause asthma [41].

4.3 Genetic background[+]

At present roughly 20% of the Western population is atopic. Predisposition of atopic constitution is considerable. If one parent is atopic the risk to be allergic is 50%; when both parents are atopic the risk is 60%. Genome-wide searches have shown that many genetic loci predispose to asthma and to date, three of such searches were described [42;43] with different results. Explanations for these differences include a variety in phenotype and the use of different ethnic groups. Genetic influences probably fall into two types. The first is the ability of the susceptible individual to recognise a common environmental allergen as foreign and initiate the allergic immune response. The second set of genetic influences regulates the overall cytokine response. Linkage of atopy to various chromosomes is reported. Total IgE concentration, the production of specific IgE, and bronchial hyperactivity are to some degree under genetic control. The regulation of total IgE concentration has been related to specific regions on chromosomes 5 and 11 in African Americans [43] and on chromosome 12 in Caucasians and Afro-Carribians [44]. Furthermore, polymorphisms of many genes, coding predominantly for cytokines, but also cell surface receptors, have been implicated to play a role (“candidate genes”). Combining chromosomal regions of interest with candidate genes

[+] Genetic background information is mainly based on the publications by D. Jarvis and P. Burney in Clinical Review, 316, 607-610, 1998 and the Editorial written by J.J. Cogswell in Clinical. and Experimental Allergy, 30; 1-3, 2000.

that reside in those regions results in “positional candidates”. Unravelling the genetic basis of asthma is made more difficult by the lack of a single clinical phenotype. Thus, the definition of the disease phenotypes is very important in the study of asthma. Intermediate phenotypes are used that allow objective measures of quantitative traits underlying the disease. These are more amenable to objective measurement than clinically diagnosed asthma. Subject selection is also important to produce valid conclusions.

4.4 Early infections

The predisposition of asthma develops early in life and childhood asthma has its onset before six years of age [45]. The actual development of asthma is seen between 5 and 11 years which declines beyond 8 years of age [20]. During pregnancy foetal immunity is dominated by humoral (T_h2) responsiveness [29;30] which is preserved in early infancy. Consequently infancy is characterised by an increased susceptibility to allergy. Infections, which promote a T_h1 phenotype, seem to afford protection against the development of asthma and allergy [46]. For example, young adults who had measles in childhood were significantly less likely to be atopic than those who had been vaccinated and/or had no measles [47;48]. Other infections (involving a T_h1 response) with micro-organisms like *Bordetella pertussis* [49], *Listeria monocytogenes* [50], *Mycobacterium bovis* [51] also suppress the T_h2 response and thus reduce the progression of allergy/asthma. *Bordetella pertussis* has, however, also been shown to act as an adjuvant of IgE-production [52]. Other infections, like respiratory syncytial virus (RSV), brucellosis, rhinovirus and *Aspergillus fumigatus* infection [53] rather induce a T_h2 response and may thus increase the prevalence of allergic disorders. For young adults who had measles and RSV in childhood is it still under debate whether such infections promote or decrease allergy [47;48;54]. In summary: infections stimulating the T_h1 or T_h2 response may suppress respectively stimulate the development of allergy and asthma.

4.5 Exposure to air pollution

A variety of epidemiological studies has shown associations between exposure to air pollution and impaired lung function, inflammatory reactions and increased prevalence of allergy [55;56]. It remains unclear how subjects may become sensitised to allergens via air pollution. It is, however, assumed that air pollution alone does not induce allergic symptoms, but subjects may be more vulnerable to combined exposure of allergens and pollutants for responding to allergens.

Indeed, combined exposure of animals to ovalbumin or pollen plus diesel exhaust particles (DEP) or exposure to house dust mite and NO_2 leads to increased antigen-specific IgE titres as compared to stimulation with the allergen alone, indicating the adjuvant activity of air polluting components [57]. Studies in healthy subjects show a significant increase in total IgE, but not IgG, IgA and IgM following nasal challenge with DEP [58]. Subjects suffering from hay-fever responded with a higher pollen-specific IgE titre induced by co-stimulation with DEP. Particles and irritating gases increase the production of the pro-inflammatory cytokines IL-6 and IL-8 and inducible NO-synthase (iNOS) in nasal lavage fluid [59] and bronchial epithelial cells in vitro [60-62].

We observed that subjects exposed to increased traffic emission exhaled larger amounts of NO (Steerenberg et al., submitted for publication). As such, air pollution may trigger

cytokine and NO release what may not only induce inflammatory effects, but also affect the T_h1/T_h2 balance in favour of the T_h2 response, predisposing for an allergic state.
In conclusion, due to the maturation steps of the immune system during the first years after birth we have to recognise a vulnerable period in which the immune system is sensitive to environmental and life-style stress. Infections and air pollution are likely to have a profound impact on the immune system during maturation.

5. Early biomarkers of allergic disorders in children•

Current understanding of the immunological mechanisms of inflammation has led to the identification of a number of biomarkers (summarised below) that can be used to evaluate the presence and severity of established allergic inflammation. With the exception of 'family history' these markers do not predict the predisposition to become sensitised to allergens. In a subsequent section (paragraph 6.6) exhaled NO is proposed as putative early marker of sensitisation.

5.1 Family history

It has long been recognised that a family history of asthma and/or allergy in young children is strongly associated with an increased risk of development of allergic diseases. Unfortunately, the value of even a carefully obtained family history as a predictive marker is limited because of low specificity. It is also often difficult to obtain a reliable family history [63]. Despite these shortcomings, family history of atopy is used widely to predict the risk in clinical settings and as a screening test to identify at-risk children in studies.

5.2 Wheezing

The nature, outcome and significance of wheezing during infancy remain unclear. Most of the children with infantile wheezing are not symptomatic in later childhood [20;63]. Martinez *et al.* [20] have demonstrated two types of infantile wheezers. Some are only transient early life wheezers, while others, named persistent wheezers, are at a high risk of developing asthma.

5.3 IgE in cord blood

The measurement of cord blood total IgE is a useful screening test [64], and this was advocated in prevention programmes [65]. A raised cord blood total IgE is a highly specific predictor for the early onset of allergic disease and even higher in combination with a family history of atopy. Some studies show, however, that elevated cord IgE was no good predictor of clinical asthma or allergic rhinitis, as assessed at the age of 4 [66-68].

5.4 Skin prick test

Sensitisation, as assessed by positive skin prick tests or presence of specific IgE antibodies, may herald the development of clinical disease. The presence of positive skin prick tests to house dust mite indicates [69] increased risk for later development of asthma.

• The paragraph on early biomarkers is mainly based on an Editorial by Arshad and Nanabhay, Clin. Exp. Allergy 29, 576-578, 1999.

5.5 Broncholaveolar lavage (BAL)

Using fibre optic bronchoscopy in adults, eosinophilic inflammation in the airways can be demonstrated even in mild asthmatics [70] but BAL can, however, not be used for screening infant wheezers.

5.6 Nasal lavage

In contrast to BAL, nasal lavage is a non-invasive technique to study effects of allergen provocation and air pollution in children and adults. Inflammatory responses can be measured by the number of inflammatory cells and immune modulating proteins e.g. ECP, MPO, IL6 and IL8 [71;72].

5.7 Eosinophilic factors

Eosinophils play a central role in allergic inflammation. Mediators released from activated eosinophils including eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil protein X (EPX) can be detected in serum of patients with asthma. Measurement of these mediators may prove to be useful to identify 'true asthma' [73]. Note that serum ECP is also raised in atopic eczema [74].

5.8 Exhaled nitric oxide (NO)

Increased exhaled NO levels are observed in patients with allergic asthma, airway infections, and allergic rhinitis [75]. In a preliminary study we observed that school children exposed to increased traffic emission exhaled larger amounts of NO (Steerenberg et al., submitted for publication). Other studies showed that atopic subjects exhaled higher amounts of NO than controls [76]. This shows that exhaled NO is a biomarker of established allergy and bronchial asthma. The potential of exhaled NO to serve as an early marker of sensitisation is described in section 6.6.

6. Exhaled NO as potential biomarker

Below in paragraph 6.1 to 6.5, the literature of exhaled NO in respiratory allergy and asthma indicates that exhaled NO may be used as marker of established allergic disease, including asthma. In the final paragraph (paragraph 6.6) it is outlined that exhaled NO has the potency to serve as a prognostic marker of allergic disease at a stage when the disease is not yet clinically overt.

6.1 Enzymatic generation of endogenous NO

NO is synthesised from L-arginine by NO-synthase (NOS). Three NOS iso-types have been described: constitutive NOS (cNOS), inducible NOS (iNOS) and neuronal NOS (nNOS). In the lungs NO functions as a bronchodilator and participates as toxic agent and modulator of the immune system. So, depending on its concentration and site of release NO retains both beneficial and adverse effects. Under physiological conditions NO is produced in airway epithelium by cNOS, but under inflammatory conditions high concentrations of NO are produced by iNOS [77]. NO is also produced by lung macrophages, stimulated neutrophils, mast cells, fibroblasts and T-lymphocytes [78]. Corticosteroids inhibit the inflammatory process via inhibition of iNOS-induction [79;80]. Finally, high concentrations of NO are produced in the nose by iNOS [78].

6.2. Exhaled NO and lung disease

NO produced in the lung is partly exhaled and can be measured in humans and animals [81-84]. Measurement of exhaled NO offers a non-invasive method to monitor airway inflammation. The method itself is simple and can be repeatedly performed -though not easily- in children and patients with airway obstruction [80;85]. Increased concentrations of exhaled NO are observed in asthmatic patients [80-91], airway infections [92;93], allergic rhinitis and bronchiectasis [94]. In contrast to asthma, exhaled NO is not increased in patients with COPD and cystic fibrosis [75;95]. Rather a decrease in exhaled NO is noted in cystic fibrosis [89] and Kartagener's syndrome [96]. Variable results were observed in COPD-patients [97] though exhaled NO was generally decreased [98;99]. Cigarette smoking induces a large decrease in exhaled NO [75] (but not of nasal NO [100]). Between asthmatics and non-asthmatics there is no difference in nasal NO concentrations [100]. Thus, exhaled NO is, no specific biomarker for asthma, but for inflammatory disease in general.

6.3. Exhaled NO and induction of iNOS

Treatment of patients with allergic asthma but not healthy subjects with glucocorticoids [101-103] and inhibitors of iNOS [101;104] induces a decrease in exhaled NO [94]. This suggests, that the NO in allergic asthmatic patients is mainly produced by iNOS and in controls by cNOS [10598]. Indeed, asthmatics show increased expression of iNOS in the airway epithelium for which presumably pro-inflammatory cytokines are responsible [106].

6.4. The early and late asthmatic response

Endogenous NO is involved and seems to inhibit the regulation of histamine- and allergen-induced bronchoconstriction [107;108]. Following exposure of asthmatics to allergens there is a progressive increase in exhaled NO during the late, but not the early response [80;109]. Atopic asthmatic children have higher exhaled NO (13 ppb) than non-atopic asthmatics (3 ppb), atopic non-asthmatic children (4 ppb) or non-atopic non-asthmatic children (3 ppb) [110] which is in agreement with results of Lanz et al. [111] who show no difference in exhaled NO between atopic non-asthmatics and controls. On the other hand Adiseh et al. showed in adults that exhaled NO was 3 times higher in symptomatic atopic subjects than in asymptomatic subjects (18 vs. 6 ppb) while nasal NO was increased, as well, in atopic subjects [112]. The latter finding is in agreement with our own studies showing higher concentrations of exhaled NO in asymptomatic atopic individuals compared to non-atopic controls [76]. In addition, preliminary results show that children with high specific IgE titres to house dust mite have significantly higher exhaled NO levels compared to non-atopic children (van Amsterdam, in preparation).

6.5. Allergic rhinitis

Exhaled NO in patients with symptomatic allergic rhinitis is significantly higher than in controls and is decreased by treatment with steroids [113]. Children with allergic rhinitis have higher nasal NO than non-atopic controls. In this study corticosteroids decreased nasal NO and there was no difference in exhaled NO between patients and controls. It is remarkable that one hour after nasal challenge with allergens nasal NO had decreased again (significant correlation with increased rhinitis symptoms) [114-116].

6.6. NO as an early predictor of sensitisation

We recently performed various studies in school children using exhaled NO as putative biomarker of adverse effects [117]. Our preliminary results from a cross-sectional study show a large variation in the level of exhaled NO. A high level of exhaled NO was defined as an exhaled NO-value exceeding at least two times the standard deviation the mean NO-level of the non-atopic children (> mean + 2x standard deviation).

Interestingly, the major part (84%) of the children with high exhaled NO showed relatively high specific IgE-antibodies titres to aero-allergens or were positive in the skin prick test, i.e. 84% of children with high exhaled NO were severely allergic. Allergic children with a relatively low antibody titre generally showed no high exhaled NO level; only 39% of allergic children (as assessed via positive skin prick test or elevated antibody titre) showed high exhaled NO. This implies that a high exhaled NO level is a reliable marker of a severe, but not of a mild, state of allergic pulmonary disease. The skin prick test or serological IgE titre assay remains the superior approach to determine an allergic status.

However, the same study shows that 2-3% of the children showed a high level of exhaled NO, but no signs of allergy (negative skin prick test, and low specific IgE-titre to aeroallergens). In addition, most of the young children (about 8 yrs) with high exhaled NO showed low specific IgE-titres to house dust mite (HDM), whereas older children (about 12 yrs.) with high exhaled NO frequently showed high specific titres to HDM. Accordingly, increased pulmonary NO-production may precede actual sensitisation and outcome of allergic

disease and symptoms. It is therefore hypothesised that increased exhaled NO at young age (not due to established allergy) may be a predictive marker of allergic disease to become manifest in following (four) years.

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Appendix 1 Mailing list

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