Thesis **Walter A.F. Balemans**

The role of childhood respiratory tract infections in the development of atopic disease

Voor mijn lieve vrouw Margreet
Ter nagedachtenis aan mijn schoonvader,
hij zou trots geweest zijn.

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The role of childhood respiratory tract infections in the development of asthma and atopic disease

De rol van luchtweginfecties op de kinderleeftijd in de ontwikkeling van astma en allergische ziekte

(Met een samenvatting in het Nederlands)

PROEFSCHRIFT

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Chapter 1

General introduction

Walter A.F. Balemans

General introduction

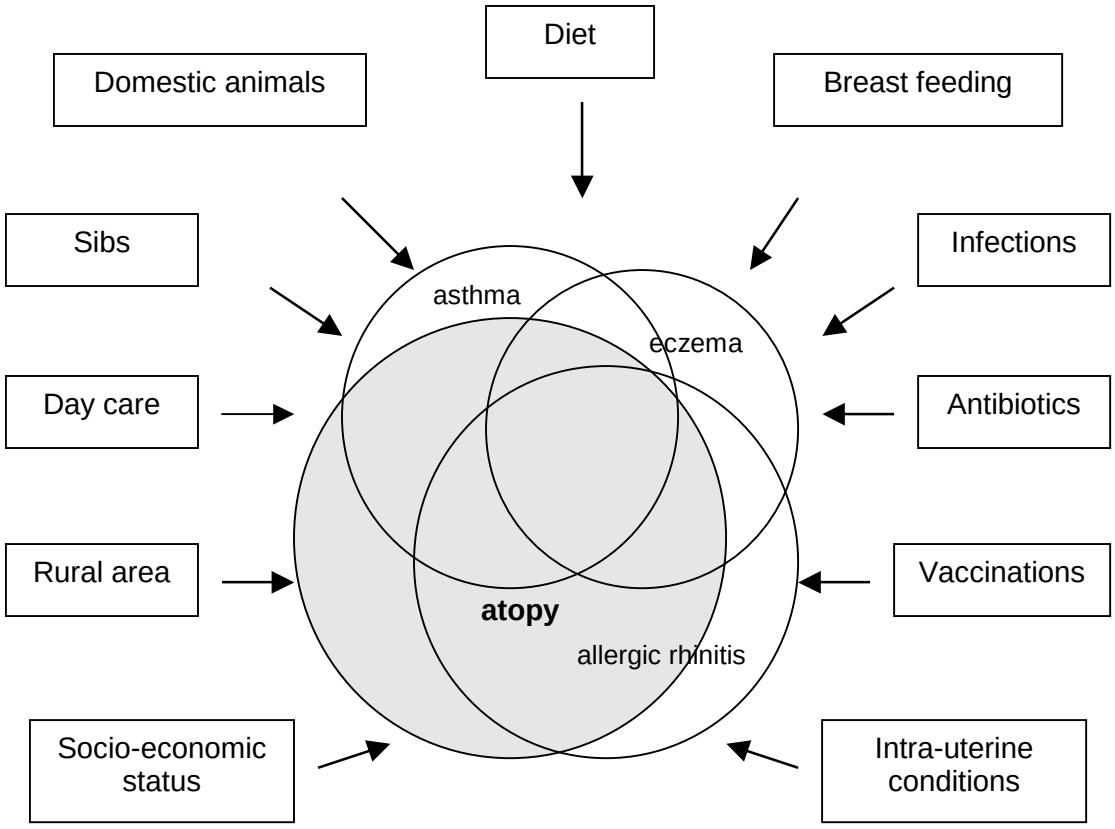
The prevalence of allergic diseases has increased over the last decades.[1-3] More recent data, however, showed no further progression and possibly even a decline in several countries.[4-12] The yearly average increase till the end of the 1990s was about 5% [13] and unlikely to be explained by genetically determined factors but rather by changing environment and lifestyle factors, like socioeconomic status, dietary habits, allergen exposure and childhood microbial exposure. (Figure 1.1)

In 1989 Strachan was the first to propose a novel but speculative explanation for the apparent rise in the prevalence of allergic diseases**:** *"These observations….could be explained if allergic diseases were prevented by infections in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally…."*[14]
In first instance, Strachan's hypothesis was received with skepticism, since up to then infections were considered to trigger allergic sensitization rather than being protective. However, during the 1990s new immunological concepts arose from studies in laboratory animals. It was recognized in rodents that the immune response against viral and bacterial infections induced a T helper 1 (Th1) pattern of cytokine release and supposed to suppress T helper 2 (Th2) immune responses involved in allergy.[15]
More recently, it became evident that the critical balance is not the Th1 versus Th2 status but rather a balance in regulatory T-cells (T_{reg}) and effector T-cells (T_{ef}) affecting both pathways.[16,17] The T_{reg}-cells are considered responsible for regulation of both $Th1_{ef}$ - and $Th2_{ef}$-cells. T_{reg}-cells are suggested to be primed via infections and specific micro-organisms in the gut and airways and to induce tolerance and anti-inflammatory action via cytokines like Il-10 and TGF-ß.[18-21] Deficient T-cell regulation may result in both auto-inflammatory disorders like inflammatory bowel disease and type I Diabetes, or atopic diseases.

Epidemiological studies on the hygiene hypothesis also showed conflicting results. Especially the role of early childhood respiratory tract infections in the development of atopic disease remains unclear. Whereas some studies did find a protective effect, others showed positive associations for respiratory tract infections and the development of asthma and atopic disease.[22-31]
Most studies performed so far, however, are hampered by methodological problems. Many have a cross-sectional or retrospective design[24-26,32], introducing all kinds of bias and unable to show time-relationships. Furthermore, most studies had a relative short follow-up.

Another major problem in epidemiological studies, is the lack of a generally accepted definition of asthma. Most epidemiological studies regarding asthma and allergy rely on individual subjective items, like ´wheezing during the last 12 months´, ´doctor's-diagnosed asthma´, and/or ´the use of asthma medication´.[33] Questions are often derived from questionnaires of international studies, such as the Core Questionnaire of the International Study of Asthma and Allergies in Childhood (ISAAC).[34]

Figure 1.1 Environmental factors influencing the development of atopic diseases



However, the validity and reliability of these questionnaire items is not really known and there are no generally accepted scoring systems either. Some studies therefore use objective measures, like lung function,
bronchial hyper responsiveness, serum IgE levels and promising new markers of airway inflammation, like exhaled bronchial nitric oxide.[35,36] The relation between these objective measures and subjective questionnaire-based items has not been studied properly. In order to gain better insight in complexes of subjective items and objective measures, factor analysis may be a useful statistical tool. Factor analysis comprises a technique to reduce multiple items and measures to a few more or less independent domains of associated variables, called factors.
The associated items in a factor proof to be more valid and reliable than the individual items as such. So far, factor analysis has only been used in cohorts of asthma patients[37] and not in general population studies. Application in a birth cohort of the general population may provide better insight in the heterogeneity of asthma and atopic disease.

For optimal prevention and treatment of children at risk of developing asthma, one would need early criteria to distinguish those children at high risk from those at low risk. Previous studies on asthma described risk factors associated with persistence or recurrence of asthma symptoms[38-41], but these risk factors are not applicable to predict the risk of an individual patient. The very few studies so far who tried to predict asthma later in life, did not develop a clinical prediction rule.[42,43]

Objectives of the current thesis

The main objective of this thesis therefore was to study the role of early childhood respiratory tract infections in the development of asthma and atopic disease in young adulthood.

Research questions were:

1. Are recurrent upper respiratory tract Infections (URTI) in childhood associated with asthma and atopic disease in early adulthood?

2. Are ENT-operations for recurrent URTI, i.e. adenoidectomy and tonsillectomy, associated with the development of asthma and atopic disease later in life?

3. Is asthma in young adults predictable in childhood on the basis of easily obtainable data like infections and parental atopic disease?

4. What is the association between FeNO and questionnaire-based diagnosis of atopic diseases, IgE and lung function measurements?

5. What are the prevalences of recurrent URTI and relapsing/persistent recurrent URTI and associated medical consumption between 0 and 21 years of age?

6. Can subjective reported items and objective measures of asthma and atopy be clustered into independent factors and how do these factors relate to each other?

Design of the study

We studied a birth cohort of the early eighties of the last century, that originally aimed to study the natural course of otitis media.[44] For this study, all children born in the city of Nijmegen between September 1982 and September 1983, were invited to participate in a study on otitis media at their second birthday (N=1439). The participating children were followed prospectively from 2 (N=1328) to 8 years (N=946).[44,45]
At age 2 years a thorough history was taken of both URTI and lower respiratory tract illness (LRTI) during the first two years of life. Baseline parameters such as duration of pregnancy, birth weight, duration of breastfeeding, parental smoking, number of siblings, day care attendance and educational level of the parents were collected. Between ages 2 and 4 years, children were visited at home every three months by a trained study nurse. At every three-monthly visit a detailed standardised interview regarding URTI and LRTI in the previous three months was taken.[44] For the current study questions, all participants were re-invited at the age of 20 to 21 years for assessment of the presence of atopic disease; the ISAAC questionnaire and additional measurements of pulmonary function (pre- and post-bronchodilator FEV_1, FEV_1%FVC), FeNO, serum IgE and Phadiatop were obtained. The results are described in this thesis.

Outline of the thesis

Chapter 2 presents an overview of existing literature on the hygiene hypothesis as well as potential reasons for the conflicting results from the various studies.
In **Chapter 3** the hygiene hypothesis is investigated in more detail by studying the association between childhood recurrent URTI and adult atopic disease measured with the ISAAC questionnaire (C*hapter 3a*) and objective measures (C*hapter 3b*). Furthermore, the association between childhood adenoidectomy and/or tonsillectomy and adult atopic disease is presented (*Chapter 3c*).
In **Chapter 4** the results of a prognostic study, in which we tried to identify children at risk for asthma in young adulthood, are presented.
The associations between FeNO and questionnaire-based diagnosis of atopic diseases, IgE and lung function measurements are described in **Chapter 5**.
Chapter 6 describes the prevalences of recurrent URTI and relapsing/persistent recurrent URTI and associated medical consumption between 0 and 21 years of age.
The results of the factor analysis and the corresponding associations between subjective and objective factors of asthma and atopy in the general population are presented in **Chapter 7**
In **Chapter 8** the main findings are summarized and discussed and some recommendations for future research are made.
Chapter 9 comprises a summary in Dutch.

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Chapter 2

The role of childhood infections in the development of asthma and atopic disease: a review of the hygiene hypothesis

Walter A.F. Balemans

The role of childhood infections in the development of asthma and atopic disease: a review of the hygiene hypothesis

Background

Over the last decades the prevalence of allergic diseases has increased [1-3], but more recent data show no further rise and possibly even a decrease in several countries.[4-12] The yearly average increase till the end of the 1990s was about 5% [13] This was unlikely to be explained by genetically determined factors, but rather by a changing environment and lifestyle factors. A striking example is increase in prevalence of atopy and allergic rhinitis within a decade in former East Germany after the breakdown of the iron curtain.[14-17]

In 1989, Strachan was the first to propose a novel but speculative explanation for the apparent rise in the prevalence of allergic diseases[18]**:** *"These observations….could be explained if allergic diseases were prevented by infections in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally…."*

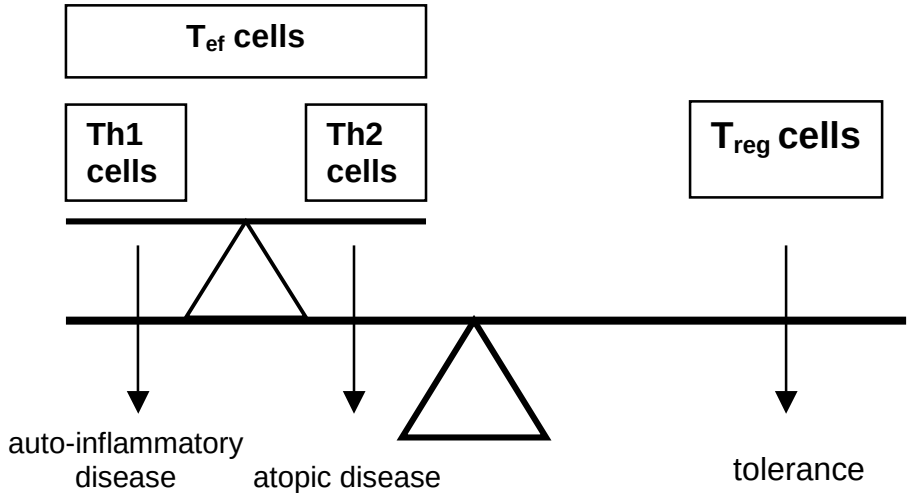
This hypothesis was, at first, received with skepticism because at that time the prevailing immunological thinking considered infection as a potential trigger of allergic sensitization rather than as a protective influence.[19,20] However, during the 1990s new immunological concepts arose from studies in laboratory animals. In rodents it was recognized that the immune response against viral and bacterial infections induced a T helper 1 (Th1) pattern of cytokine release, thought to suppress T helper 2 (Th2) immune responses involved in allergy.[21] More recently it became evident that the critical balance is not the Th1 versus Th2 status as such, but rather the status of regulatory T cells (T_{reg}) and effector T cells (T_{ef}) which affect both pathways.[22,23] (Figure 2.1) The T_{reg} cells directing both $Th1_{ef}$ - and $Th2_{ef}$ pathways are described to be involved in diseases like auto-inflammatory disorders and type I Diabetes and also atopic diseases. T_{reg} cells are suggested to be primed via infections and contacts with micro-organisms in the gut and airways and to induce tolerance and anti-inflammatory action via cytokines like Il-10 and TGF-ß.[24-27] Atopic disease and allergy may thus result from defective T-cell regulation resulting from changed environmental conditions, like a decrease in early childhood infections.

After Strachan, many epidemiological studies emerged about the issue of the hygiene hypothesis but with conflicting outcomes. In this chapter, we discuss epidemiological studies pointing at a possible role of environmental factors in the development of atopic diseases with the main focus on the effect of early childhood respiratory tract infections. Potential reasons for the conflicting results of the various studies are discussed.

The changing environment

Many epidemiological studies have described factors potentially influencing microbial exposure in early life which have changed in the last century and that might explain the apparent inverse relation between infectious burden in early life and the development of atopic disease. The most important candidate factors are enumerated in Figure 1.1 of Chapter 1.

Figure 2.1 The balance of T regulatory cells (T_{reg}) and T effector cells (T_{ef})



Socioeconomic status
Already in the 19th century Blackley described that hay fever was a disease of the urban educated classes and was rare among farmers, despite their high exposure to pollen. Since Strachan, who also described an inverse relation between socioeconomic class and hay fever[18], many other studies have reported higher prevalence of hay fever[28-30], eczema[31] and atopy[32-34] among children and adults from more affluent families. This seemed independent of family size and birth order[29] and is particularly apparent with variation in parental socioeconomic status and, remarkably, not with the offspring's own socioeconomic status later in adult life.[35]

Rural areas
Several studies reported a reduced prevalence of hay fever and atopy among children of farmers compared with other children from rural areas.[36-40] A higher exposure at farms to endotoxines, which are elements of cell walls of gram-negative micro-organisms, was suggested to be responsible for the protective effect. Of interest is the potential role of the innate immune system that can interact with microbes through evolutionary well preserved pathogen-associated molecular patterns (PAMPs) such as endotoxines of gram-negative bacteria. For instance, Toll-like receptors (TLRs) and CD14 molecules of the innate immune system are expressed on human intestinal epithelial cells, and recognize PAMPs such as those of endotoxines. A lack of stimulation with PAMPs through TLRs and CD14 was suggested to be the reason for defective T-cell regulation and the increased risk of development of both allergic and auto-inflammatory disease in the non-rural society.[41-43]

Siblings and day care
A rather consistent finding noted in many epidemiologic surveys is the inverse relation of allergen sensitization to the number of siblings in the family. This phenomenon, described as the 'sibling effect', was first reported in a cross

sectional analysis of a national birth cohort in Britain.[44] With an increasing number of siblings, a significant decrease in risk of eczema and hay fever was detected. This association was later also observed in many other studies, not only for eczema and hay fever, but also for asthma, wheezing as well as sensitization to allergens.[45] Moreover, many studies found a stronger protective influence of older compared to younger siblings.[33,46-47] One study found an additional independent protective effect of sharing a bedroom.[48] In a systematic review on the 'sibling effect' by Karmaus and Botezan of all reports between 1965 and 2000.[48] 10 out of 11 reviewed studies reported an inverse association between the number of siblings and eczema[30,44,48-54],17 out of 17 studies reported an inverse association between the number of siblings and hay fever[30,36,44,46,49-62],14 out of 16 studies reported an inverse association with sensitization to allergens[17,33,34,46,47,48,57,58,63-68],and 22 out of 31 studies reported an inverse association with asthma or wheezing.[36,49,50,52,53,55-60,62,69-84] and thus confirm rather consistently the sibling effect thought to represent increased exposure to microbes early in life.

Another specification of the hygiene hypothesis is exposure to other children in day care and nursery facilities. There is good evidence that day care attendance increases the number of infections a child suffers[85-90], particularly of the respiratory tract.[91] Attending day care, especially at young age[92], might therefore have a similar protective effect as having siblings. However, both negative[68,80,92-96] and positive[47,48,54,97,98] associations with atopic disease have been reported.

Antibiotics

The use of antibiotics is another potential risk factor of interest altering microbial exposure. Although a series of epidemiologic studies have supported[98-103] an association between antibiotic use in childhood and asthma and atopic disease, others refuted this association[104,105]. The effect was suggested to be a direct effect of antibiotic use by altering the intestinal flora and diminished Th1 immune responses.[106] Antibiotic use might however also be a proxy marker for childhood infections. So far, it is not clear whether there exists a causal relation between the use of antibiotics and asthma and atopic disease.

Childhood respiratory tract infections

Socioeconomic status, family size and day care attendance and even antibiotic use are all indirect markers of infectious burden. Research has not yet answered the question what causal factor actually explains the "sibling - and day care effect" and some authors suggest that respiratory infections, especially at early age, might be the real reason.

The first reports suggesting an inverse link between respiratory tract infections and asthma were observational studies in two island communities, Tristan da Cunha in the Atlantic and the West Carolina islands in the Pacific. In these isolated populations the incidence of respiratory virus infections is very low, whereas the prevalence of asthma is extremely high.[107,108] Although this might suggest a protective effect of respiratory infections, genetic influences in these isolated communities may very well confound the association.

Another striking observational study was reported by von Mutius et al.[16] after the re-unification of Germany in 1990. In a genetically homogeneous

population, which had been separated by the “iron curtain” for more than 40 years into East and Western communities, a higher prevalence of atopy and asthma in former West Germany compared to former East Germany among 9 to 11 year old children was observed. They suggested these differences to be due to a higher incidence of respiratory infections in East Germany, because of overcrowding, larger families and widespread use of day care. Remarkably, after the re-unification a rapid increase of hay fever and atopic sensitization was observed in the former East German Republic.[109] Although these findings are at least highly suggestive for a causal factor in Western lifestyle, they provide no proof for a direct role of respiratory tract infections in particular.

Only few studies looked carefully into the role of childhood respiratory tract infections in the development of atopic disease (Table 2.1), but also showing conflicting results. Although not consistent, many studies describe a positive relation between symptomatic respiratory tract infections and atopic disease, but report an inverse relation between indirect markers of infectious burden, like siblings and day care attendance.[45,58,103,110,111] For example, in a Finnish study an inverse relation between the number of siblings and asthma was found, but parental reports on the number of respiratory infections were positively associated with having asthma.[54] Another example of this paradox is described in an Australian study on data regarding family size and upper and lower respiratory tract infections in the first year of life in a birth cohort.[58] These authors confirmed an inverse relation between family size and asthma at age 7 years but found a positive association between both upper and lower respiratory tract infections in the first months of life and asthma at age 7 years.

Non-respiratory infections and vaccinations

The observation that measles might be protective against allergy is of great interest. Among children in Guinea-Bissau, after an epidemic of wild measles in young, non-vaccinated children, a substantially reduced prevalence of allergic sensitization (SPT positive) was observed in the group of infected children compared with those who were vaccinated after the epidemic.[112-114] The authors concluded that measles infection might protect against atopy, or that vaccination against measles might increase the risk for atopy. Other studies, however could not confirm a protective effect of wild measles[44,115,116], nor an effect of vaccination.[113,115]

Modern vaccines administered in infancy may induce a more Th2-like vaccine responses [118-120] Concerns have therefore been raised that vaccinations early in life may induce allergy. These concerns were supported by some experimental studies in animals[121,122] Furthermore, retrospective studies found a lower prevalence of atopy among children with a low vaccination coverage level.[78,123,124] However, in communities with a high vaccination level, unvaccinated families are most likely not representative for the population as a whole. Furthermore, prospective trials, for example with acellular pertussis vaccine which primary induces a Th2-like response, showed no support of the hypothesis that vaccines promote development of atopy or asthma.[125-127] In fact, one prospective study even showed a transient negative association between cumulative immunization coverage and atopy.[128]

Table 2.1 Epidemiologic cohort studies into the relation of childhood respiratory tract infections and subsequent atopic disease

First author Country Study year	Study type Follow-up (y*,mo[#])	Sample size	Infection type Exposure age	Outcome measure Age (y*, mo[#])	Results	Ref nr[$]
Castro-Rodriguez J USA 1980-'84	prospective follow-up 0-13y	1.246	every episode of lower respiratory tract illness (physical exam, viral culture) at age 0-3 y	asthma (questionnaire; lung function) at age 6 and 11 y; atopy (IgE) at age 9 mo, 6 y, 11 y	positive association LRTI[&] and pneumonia with asthma at 6 and 11 y; inverse relation non-wheezing LRTI and IgE	80, 110
McDade T USA 1983	prospective follow-up pregnancy – 15 y	99	diarrhoea and respiratory infection (home visit every 2mo) at age 0-1 y	IgE at age 14-15 y	inverse association episodes of infection first 6 mo and IgE level at 14-15 y	167
Ponsonby A Australia 1988	prospective follow-up 0-7 y	863	respiratory infections at median 35 d (home visit) and 85 d (telephone interview); family size at first months	asthma and hay fever (questionnaire) at age 7 y	positive association URTI[%] and LRTI first year with asthma; inverse association resident number with asthma and hay fever	58
Oddy W Australia 1989-'92	prospective follow-up 0-6 y	2.602	respiratory infections (diary and questionnaire) at age 1 y	asthma (questionnaire), atopy (SPT[†]) at age 6 y	1-3 URTI protective, >3 URTI risk factor for asthma; no association URTI and LRTI and atopy	168
Illi S Germany 1990	prospective follow-up 0-7 y	1.314	viral infections, resp infections (interview and diary) at age 1,3,6,12,18 mo and then yearly till age 7 y	asthma (questionnaire), atopy (SPT), AHR[‡] (histamine challenge) at age 7 y	inverse association ≥2 runny nose and asthma, wheeze or atopy ; inverse association ≥1 herpes infection and asthma; positive association LRTI and asthma;	104
Nafstad P Norway 1992-'93	prospective follow-up 0-10 y	2.450	respiratory infections 0-1 yrs, day care, birth order (questionnaire) at age 6 and 12 mo	asthma (questionnaire), atopy (SPT) at age 10 y	positive association LRTI, croup, otitis first year with asthma; no association URTI and LRTI with hay fever; no association birth order and day care	169
Nystad W Norway 1994	Retrospective	1.447	respiratory infections, day care (questionnaire)	asthma (questionnaire) at age 6-16 y	positive association infections and day care, infections and asthma	97

Table 2.1 Continued

First author Country Study year	Study type Follow-up (y*,mo#)	Sample size	Infection type Exposure age	Outcome measure Age (y*, mo#)	Results	Ref nr$
Kilpi T Finland 1994	prospective follow-up 2 mo-2 y	329	respiratory and viral infections, AOM (interview, physical exam, viral culture (PCR$^{\Delta}$)) at age 2,3,4,5,6,9,12,15,18,24mo	eczema (physical exam) at age 2 mo-2 y	protective effect of respiratory and viral infections on eczema	170
Pekkanen J Finland 1994-'95	retrospective	8.387	number of sibs, birth order, day care, respiratory infections on average (questionnaire) at age 1-3 y	asthma, hay fever, eczema (questionnaire) at age 13-14 y	no association birth order, day care and atopic disease; negative association sibs and asthma, hay fever and eczema; positive association respiratory infections on average and asthma	54
Cohet C New Zealand 1994-'95	retrospective age 0-7 y	1584 + 2539	confirmed serious infections (national database), siblings, antibiotics, paracetamol (questionnaire) at age 0-4 y	asthma, hay fever, eczema (questionnaire) at age 6-7 y	no difference between atopic disease in infection group compared to controles in general population; positive association with antibiotics, paracetamol and asthma, hay fever and eczema	171
Farooqi I UK 1996	retrospective age range 12-21 y	1.934	immunizations, doctor's visits for infections, antibiotics, siblings (public health and general practice records) at age range 0-21 y	asthma, hay fever, eczema (general practice records) at age range 2-21 y	positive association pertussis vaccination and all sorts of infections with asthma and any atopic disease; no association siblings; positive association antibiotics; independent risk factors for asthma: pertussis vaccination and antibiotics	99
McKeever T UK 2001	prospective follow-up median 2.9 y (range 0-11y)	29.238	doctor's visits for infections (respiratory, viral, bacterial, gastro-intestinal), antibiotics, birth order (general practice database) at age 0-1 y	asthma, hay fever, eczema (general practice database) from 2 y to end of follow-up, median age 2.9 y (range 0-11)	inverse association birth order and asthma, hay fever and eczema; slightly positive association with infections (less apparent after adjusting for consulting behaviour); positive association respiratory infections and asthma	103, 111

*y: year; #mo: month; $Ref nr: reference number; %URTI: upper respiratory tract infections; &LRTI: lower respiratory tract infections; †SPT: skin prick test; ‡AHR: airway hyper responsiveness; ΔPCR: polymerase chain reaction

Another infection of interest is chronic infection with mycobacterium tuberculosis. However, where some cross-sectional studies showed an inverse association with atopic disease[129,130], others did not confirm this association[131]. Results on a potential effect of BCG vaccinations are conflicting as well.[132]

Perhaps the most consistent and convincing evidence of an inverse relationship between infection and allergic sensitization has emerged from studies of hepatitis A. Matricardi et al. reported an independent inverse association between serologic evidence of a previous infection with hepatitis A and sensitization to aero-allergens among Italian military recruits.[66] In the same study cohort, there was also an inverse relationship with seropositivity to *Toxoplasma gondii* but not with positive serology for measles, mumps, rubella, varicella, cytomegalovirus, herpes simplex or *Helicobacter pylori*.[133] A few other cross-sectional studies confirmed the negative relationship with hepatitis A, but the lack of timeframe in these cross-sectional studies makes it impossible to determine the direction of the association.[81,134] A novel finding is the TIM-1 gene, which is the first molecular genetic link with the hygiene hypothesis. TIM is a T-cell immunoglobulin domain gene, encoding transmembrane proteins of CD4 positive T cells, and shown to play a critical role in regulation of the development of atopic disease in a mouse model. In humans, certain polymorphic variants of TIM-1 are associated with protection against atopy in individuals who have had an infection with hepatitis A. Since TIM-1 functions as the receptor for hepatitis A virus on T-cells, it is postulated that infection with hepatitis A may affect T-cell differentiation, and reduces Th 2-driven inflammatory responses.[135,136]

In general, the prevalence of atopic diseases is greater in industrialized countries compared with non-industrialized countries.[137,138] Moreover, in many African countries the prevalence is very low, but greater among populations with a more urbanized lifestyle.[139] Geohelminth infections, like *Ascaris lumbricoides*, *Trichuris Trichiura* and *Ancylostoma duodenale*, are probably the most prevalent and persistent of all childhood infections in non-industrialized countries.[140] Several studies have shown a negative association between infections with these parasites and atopy[141-143] and wheezing.[144] The concept of geohelminth infections being protective against atopy is also supported by a study describing an increasing prevalence of atopy after long-term antihelmintic treatment.[145]

Conflicting evidence

Although the hygiene hypothesis has been favoured since the early nineties of last century, a lot of criticism has emerged during last years. The paradox of positive associations between measured symptomatic respiratory tract infections and atopic disease but inverse associations with proxy markers is complex, and many reasons may be responsible for the contradiction. The evidence in favour of the hygiene hypothesis is mainly based on cross-sectional studies with a lack of information on timing of exposure and outcome relationships, which makes it hard to draw firm conclusions about the direction of a certain association. Furthermore, observational studies are susceptible to

confounding, selection, and information bias, which will be described in more detail below.

Selection bias

There is increasing evidence that asthmatics and atopic individuals suffer from more severe symptoms of the same respiratory infections compared to non-atopic individuals.[146] There are suggestions that in asthmatics viral replication is increased and prolonged, due to defective Th1 immune response against viruses.[147-149] Studies using surrogate markers measure exposures independent of the host response to this exposure and may often find a protective effect in accordance with the hygiene hypothesis. In studies, however, actually counting symptomatic respiratory infections, the response of the individuals to these infections may bias the outcome, since individuals at high risk of atopic disease are more likely to develop symptoms with viral respiratory tract infections. In other words, studies in which symptomatic infections are measured may select those subjects at high risk of developing asthma and atopic disease.

Other examples of selection bias, influencing the negative association between living on a farm and atopic disease, are the fact that allergic individuals are less likely to succeed their father as a farmer[150] or behaviour of the parents. Selection over many decades of the individuals that fit genetically in the farm environment may explain the negative association between living on a farm and atopic disease. Also, parents of children with recurrent lower respiratory symptoms may be more reluctant to send their children to a nursery, which in turn leads to an apparent protective effect of day care attendance.

Confounding

Many studies do not adjust for potential confounding factors, like breastfeeding, exposure to tobacco smoke, birth-weight, gestational age, perinatal exposure and parental atopy.[151,152] For example, fever and flu episodes in pregnancy, threatened abortion, intra-uterine growth and delivery duration, may act as potential confounders since they may be associated with both the determinant (respiratory tract infections) and the outcome (asthma or atopic disease)[153-160]

Furthermore, studies using surrogate markers measure overall exposure of infectious burden, which could be biased by other infections or exposures of other origin that are not measured. A good example of these are gastrointestinal infections, which are proven also to occur with a higher frequency at day care centres[161] and may have a protective effect on atopic development.[162]

Another form of confounding is called confounding by indication, which plays a role in studies that reported on the association between antibiotics and asthma. Antibiotics are often prescribed for a reason, e.g. symptoms of the lower respiratory tract. These symptoms may be the early manifestations of asthma. By comparing children who were and were not prescribed antibiotics, actually children with and without a certain disease or symptoms are compared. Celedon et al. described this in a well-designed prospective cohort study in which they showed that the association of antibiotic use among children with asthma symptoms in the first two years became weaker after adjustment for lower respiratory infections or General Practitioner consulting behaviour.[163]

Information bias

Information bias might occur if the determinant and the outcome are not classified correctly.

Since the determinant, i.e. respiratory infections, is often mild and self-limiting for which no doctor's advice is sought, recall bias might occur. For example, upper respiratory tract infections with concomitant symptoms of the lower respiratory tract, like wheeze, dyspnoea or serious cough, are probably remembered, whereas a runny nose is easily forgotten.

The outcome, i.e. diagnosis of asthma, is often made on the basis of questionnaires, and different studies appear to use different items. It is well known that respiratory symptoms in childhood, like wheezing and coughing, are mainly based on viral infections. Only a minority of asthmatic symptoms are attributable to atopic asthma.[164] Asthma is probably more likely to be misdiagnosed than allergic rhinitis, which has more clear and specific symptoms.[45] Some authors therefore plead to define asthma in epidemiologic studies on a combination of symptoms and bronchial hyper responsiveness.[165] However, up till now no generally accepted definition of asthma is available and no validated quantitative criteria are standardized for clinical and epidemiological purposes. Furthermore, studies differ in follow-up time. Adults with asthma may have another phenotype than children with asthma. For example, in the Belmont cohort was shown that adults with asthma symptoms did not have manifestations of the disease at 8 – 12 years of age.[166] Many individuals develop atopy and subsequent asthma after school age and these individuals may have a different asthma phenotype with different risk factors compared to individuals with childhood asthma or persistent asthma from childhood through adulthood.[166]

Conclusions

Summarizing, the evidence in favour of the hypothesis of childhood infections to protect against atopic development is merely based on studies using indirect markers of overall microbial exposure, i.e. crowding of children. There is no strong evidence that early and symptomatic childhood respiratory tract infections as such protect atopic development. Most studies that measured symptomatic respiratory infections in childhood even found a positive association between early infections and atopy, which might be due to reversed causation, i.e. individuals with a genetic predisposition to develop atopy and asthma are at increased risk to develop respiratory symptoms early in life. Several types of bias, i.e. confounding, selection and information bias may cause the conflicting results so far. To solve the paradox we need longitudinal birth cohort studies with detailed prospective documentation of childhood respiratory infections, assessment of potential confounders, and extended follow-up time.

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Chapter 3 A

Recurrent childhood upper respiratory tract infections do not reduce the risk of adult atopic disease

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Recurrent childhood upper respiratory tract infections do not reduce the risk of adult atopic disease

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Abstract

Background
Children of large families and those attending day care are at increased risk of respiratory tract infections, which in turn may protect against the development of allergic disease. Longitudinal studies investigating these associations beyond childhood are, however, scarce.

Objective
To investigate the association between childhood recurrent upper respiratory tract infections and asthma, allergic rhinitis, and eczema in adulthood.

Methods
A birth cohort of 1055 members followed prospectively from ages 2 to 21 years. Detailed information on upper respiratory tract infections (URTI) between ages 2 and 4 years was collected at three-monthly intervals in a standardised interview. At age 8 years a parental questionnaire regarding URTI between ages 4 and 8 years was used. The incidence of asthma and atopic disease at age 21 years was determined, using a standardised questionnaire.

Results
Of the original cohort, 693 (66%) members completed the questionnaire. Children who experienced recurrent URTI before the age of 2 years, between ages 2 - 4 years and between ages 4 - 8 years were not less likely to have asthma at age 21 years than children who did not experience recurrent URTI; relative risk (RR) 0.97 (95% CI 0.65 - 1.46), RR 1.45 (CI 0.95 - 2.21) and RR 1.51 (CI 0.97 - 2.36), respectively. Neither were recurrent URTI associated with a decreased risk of allergic rhinitis, nor eczema at the age of 21 years.

Conclusions
Recurrent URTI in childhood did not reduce the risk of atopic disease in young adulthood.

Introduction

Atopic disease is an increasing health problem in Western societies.[1] The reasons for this rising trend are not yet clarified, but environmental factors are likely to be important. Strachan was the first to report the association between high socio-economic status and low number of siblings on one hand and high frequency of hay fever on the other hand. This finding resulted in the so-called 'hygiene hypothesis', which proposes a protective effect of childhood infections on the development of atopic disease.[2] Although the exact mechanisms are not yet fully understood, it is suggested that infections direct the immune system towards Th1-like responses, whereas atopic disease is related to Th2-like responses. Since Strachan, the association between decreased infectious environmental burden and increased development of atopic disease has been confirmed in numerous studies using various markers such as family size, older siblings, day care attendance and exposure to livestock on farms.[3-7]
Upper respiratory tract infections (URTI) are the most common infections in children, and children of large families and those attending day care are at increased risk of respiratory infections.[8,9] The role of these infections in the development of asthma, however, is still controversial. Both positive as well as negative associations between URTI and asthma have been reported.[10-12] So far, most studies on this topic have been retrospective in design, which might induce recall bias. Some have used doctor-diagnosed URTI in their analyses, whereas most URTI are known to be self-limiting and no medical advice is sought. Finally, in most studies asthma was diagnosed at young age when it is still difficult to distinguish from recurrent viral wheeze. In this prospective follow-up study of a large birth cohort, followed up from 2 to 21 years of age, we investigate the association of documented recurrent respiratory tract infections in childhood and adult atopic disease.

Methods

Study population and follow up
All children born in the city of Nijmegen between September 1982 and September 1983 , were invited to participate in a study on otitis media at their second birthday (N=1439). Of this birth cohort 1328 subjects have been followed prospectively from 2 to 8 years; detailed parental reports on URTI during this period are available.[13,14]
At age 2 years a thorough history was taken of both URTI and lower respiratory tract illness (LRTI) during the first two years of life. Baseline parameters such as duration of pregnancy, birth weight, duration of breastfeeding, parental smoking, number of siblings and educational level of the parents were collected. Between ages 2 and 4 years children were visited at home every three months by a trained study nurse. At every three-monthly visit a detailed standardised interview regarding URTI and LRTI in the previous three months was taken and tympanometry was performed.[13] At the age of 7 to 8 years children (N=1006) were re-evaluated by means of a standardised questionnaire regarding URTI that occurred between ages 4 and 8 years.[14] At the age of 21 years participants were invited for assessment of the presence of atopic disease. Via the City Council of Nijmegen the home addresses were traced and cohort members

were sent another standardised questionnaire.[15] The study was approved by the local ethical committee of the University Medical Centre Utrecht, and all subjects gave written informed consent to participate in the study.

Asthma, allergic rhinitis and eczema at age 21 years
The ISAAC Core Questionnaire was used which has been validated to obtain data on asthma, allergic rhinitis and eczema.[15] Furthermore, we obtained data about current use of asthma medication, smoking behaviour in the last year and parental history of atopic diseases. The latter was defined as asthma, allergic rhinitis or eczema in at least one parent. All questions came from questionnaires used in international studies.[16,17]
Asthma at age 21 years was defined as the occurrence of wheezing in the last twelve months and/or a doctor's diagnosis of asthma in that same period and/or current use of asthma medication.
Severe asthma at age 21 years was defined as at least 4 wheezing attacks in the last 12 months and/or one or more nights per week, on average, of sleep disturbance due to wheezing.
Allergic rhinitis at age 21 years was defined as the presence of a runny or blocked nose without having a cold or flu in the last 12 months, accompanied by itchy-watery eyes and/or some interference of this nose problem with daily activities.
Eczema at age 21 years was defined as itchy skin rash that has been coming and going for at least six months and affecting particular skin sites (i.e. folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes) in the last twelve months.

Recurrent childhood upper respiratory tract infections
Recurrent upper respiratory tract infections before the age of 2 years ($URTI_{0-2}$) were defined as the occurrence of two or more ear infections (otalgia with fever and/or otorrhoea) and/or two or more throat infections over that period and/or at least five episodes of a runny nose in the last twelve months.
Between ages 2 and 4 years the number of three-monthly episodes in which an upper respiratory tract infection had occurred was counted. Recurrent upper respiratory tract infections between ages 2 and 4 years ($URTI_{2-4}$) were defined as at least three episodes in which an ear infection had occurred (otalgia with fever and/or otorrhoea) and/or at least three episodes in which a throat infection had occurred and/or at least six episodes in which a runny nose had occurred.
Recurrent upper respiratory tract infections between ages 4 and 8 years ($URTI_{4-8}$) were defined as the occurrence of two or more ear infections (otalgia with fever and/or otorrhoea) and/or two or more throat infections and/or five or more episodes of runny nose. The cut-off points were based on the median distribution of each type of respiratory tract infection at each assessment.
Antibiotic treatment for URTI before the age of 2 years, between the ages 2 and 4 years and between the ages 4 and 8 years, respectively, was defined as at least one antibiotic course in the corresponding period.
As an additional marker for URTI between ages 2 and 4 years, tympanograms, obtained at the scheduled three-monthly visits, were analysed. The number of bilateral flat type B tympanograms, as indicator of middle ear effusion (Jerger classification[18]), were calculated. Frequent middle ear effusion was defined as at least three episodes with bilateral type B tympanograms.

Childhood lower respiratory tract illness
Lower respiratory tract illness before the age of 2 years ($LRTI_{0-2}$) was defined as at least one doctor's visit over that period because of pneumonia and/or dyspnoea and/or asthma and/or bronchitis. Lower respiratory tract illness between ages 2 and 4 years ($LRTI_{2-4}$) was defined as a doctor's visit during any three-monthly episode because of pneumonia and/or dyspnoea and/or asthma and/or bronchitis. At the age of 7-8 years no history of lower respiratory tract illness between ages 4 and 8 years was taken.

Statistical analysis
We calculated 2*2 tables to analyse the effect (rate ratio) of URTI, LRTI, antibiotic treatment for URTI, type B tympanograms and number of siblings on asthma, allergic rhinitis and eczema at age 21 years. To study a possible u- or v-shape association, we categorised subjects in a group of low, intermediate and high frequency of URTI and analysed the effect of each category.
Mantel-Haenszel analyses were performed to adjust for potential confounders as well as to study possible effect modifiers, e.g. parental smoking, parental atopic disease, parental educational level, breastfeeding, birth weight and gestational age. The final rate ratios were adjusted for maternal smoking and parental atopic disease as these factors were indeed found to be confounders. Sensitivity analyses were performed to study the effect of different cut-off points. We used SAS software (version 8.0) for all statistical analyses.

Results

Addresses of 1055 subjects (79%) of the 1328 original cohort members could be traced; 693 (66%) of these 1055 subjects responded by returning the questionnaire.Baseline characteristics are shown in table 3.1. Of the 693 respondents 319 (46%) were male, 299 (44%) had a mother who smoked, and 315 (46%) had at least one atopic parent. Participating subjects were comparable to non-participating subjects according to the most important baseline parameters.
Of the 693 participants, 297 (45%) met the criteria of recurrent URTI before the age of 2 years; 314 (49%) between ages 2 and 4 years, and 265 (44%) between ages 4 and 8 years.
One or more antibiotic treatments for URTI were received by 148 (21%) participants before the age of 2 years; 269 (42%) between ages 2 and 4, and 142 (24%) between ages 4 and 8 years. Furthermore, 138 participants (22%) had three or more bilateral type B tympanograms between ages 2 and 4 years.
At age 21 years, 86 (12%) of the 693 subjects who participated in the follow up had asthma and 27 (4%) severe asthma according to our definition; 214 subjects (31%) met the criteria for allergic rhinitis and 98 (14%) for eczema.

Association between childhood respiratory tract infections and atopic disease
Table 3.2 shows the relative risks of asthma, allergic rhinitis and eczema at age 21 years adjusted for confounding factors, i.e. maternal smoking and parental atopic disease. There was no significant association between $URTI_{0-2}$, $URTI_{2-4}$ and $URTI_{4-8}$ and asthma; relative risk (RR) 0.97 (95% confidence interval (CI) 0.65 - 1.46), RR 1.45 (CI 0.95 - 2.21) and RR 1.51 (CI 0.97 - 2.36), respectively.

Table 3.1 Characteristics study population (N=693) as compared to the non-participating subjects (N=635)

	Study population N (%)	**Non participants** N (%)
Male gender	319 (46)	347 (54)
Breast fed > 3 months	222 (34)	180 (32)
Family size		
Siblings	439 (68)	389 (69)
≥ 1 older sib	378 (55)	297 (52)
Day-care 0-2 years		
≥ 2 days per week	60 (14)	51 (16)
Smoking mother	299 (44)	240 (40)
Educational level mother		
Low	278 (40)	319 (50)
Medial	257 (37)	182 (29)
High	158 (23)	134 (21)
Atopic parent(s)	315 (46)	--
Recurrent URTI		
Age 0-2 years	297 (45)	253 (43)
Age 2-4 years	314 (49)	217 (38)
Age 4-8 years	265 (44)	--
LRTI		
Age 0-2 years	84 (13)	98 (15)
Age 2-4 years	146 (21)	115 (18)
Antibiotic treatment		
Age 0-2 years	148 (21)	126 (20)
Age 2-4 years	269 (42)	208 (37)
Age 4-8 years	142 (24)	--
Bilateral type B tympanogram		
≥3 at age 2-4 years	138 (22)	--
Outcome at age 21 years		
Asthma	86 (12)	--
Severe asthma	27 (4)	
Allergic rhinitis	214 (31)	--
Eczema	98 (14)	--

Table 3.2 Relative risks (95% confidence intervals) of asthma, allergic rhinitis and eczema in 693 subjects at age 21 years according to recurrent upper respiratory tract infections, lower respiratory tract illness, older siblings and antibiotic treatment

	Asthma	Allergic rhinitis	Eczema
Recurrent $URTI_{0-2}$	0.97 (0.65–1.46)	0.89 (0.71–1.12)	1.06 (0.73–1.55)
Runny nose ≥ 5 episodes	1.00 (0.66–1.51)	0.98 (0.78–1.23)	1.19 (0.82–1.75)
Ear infection ≥ 2 episodes	1.45 (0.96–2.20)	0.96 (0.74–1.24)	1.13 (0.75–1.70)
Throat infection ≥ 2 episodes	1.02 (0.44–2.35)	0.66 (0.36–1.22)	0.97 (0.42–2.24)
Recurrent $URTI_{2-4}$	1.45 (0.95–2.21)	0.99 (0.79–1.25)	1.19 (0.81–1.75)
Runny nose ≥ 6 episodes	1.48 (0.98–2.24)	0.96 (0.76–1.21)	1.12 (0.76–1.64)
Ear infection ≥ 3 episodes	0.82 (0.35–1.92)	0.69 (0.40–1.19)	1.09 (0.54–2.20)
Throat infection ≥ 3 episodes	1.32 (0.72–2.41)	1.37 (1.01–1.85)	1.48 (0.89–2.49)
Bilateral type B tympanogram ≥ 3 episodes	0.71 (0.39–1.30)	0.72 (0.52–0.98)	1.18 (0.76–1.82)
Recurrent $URTI_{4-8}$	1.51 (0.97–2.36)	1.08 (0.85–1.38)	1.23 (0.82–1.84)
Runny nose ≥ 5 episodes	1.19 (0.73–1.94)	1.07 (0.82–1.39)	1.25 (0.82–1.92)
Ear infection ≥ 2 episodes	1.22 (0.74–2.01)	1.13 (0.85–1.49)	1.38 (0.88–2.16)
Throat infection ≥ 2 episodes	1.42 (0.81–2.51)	1.06 (0.76–1.49)	1.03 (0.58–1.84)
$LRTI_{0-2}$	1.61 (0.96–2.69)	0.74 (0.49–1.12)	1.21 (0.72–2.05)
$LRTI_{2-4}$	1.91 (1.26–2.87)	0.97 (0.74–1.28)	1.52 (1.02–2.27)
Older siblings	1.30 (0.86–1.96)	0.98 (0.79–1.23)	1.38 (0.93–2.04)
Antibiotic treatment for			
$URTI_{0-2}$	1.45 (0.94–2.26)	0.98 (0.74–1.29)	1.20 (0.78–1.85)
$URTI_{2-4}$	1.56 (1.04–2.34)	0.97 (0.77–1.22)	0.87 (0.58–1.29)
$URTI_{4-8}$	1.01 (0.60–1.68)	1.33 (1.04–1.71)	1.28 (0.82–1.98)

Adjusted for maternal smoking and parental atopy. Recurrent upper respiratory tract infections between 0 to 2 years ($URTI_{0-2}$), between 2 to 4 years ($URTI_{2-4}$) and between 4 to 8 years ($URTI_{4-8}$);
Lower respiratory tract illness between 0 to 2 years ($LRTI_{0-2}$) and between 2 to 4 years ($LRTI_{2-4}$).

Analysis of associations between $URTI_{0-2}$, $URTI_{2-4}$ and $URTI_{4-8}$ and severe asthma did not change this finding; RR 0.66 (CI 0.30 – 1.47), RR 1.17 (CI 0.56 – 2.46), and RR 2.41 (CI 0.98 – 5.97), respectively. Neither could we find an association between $URTI_{0-2}$, $URTI_{2-4}$ and $URTI_{4-8}$ and allergic rhinitis; RR 0.89 (CI 0.71 – 1.12), RR 0.99 (CI 0.79 – 1.25) and RR 1.08 (0.85 – 1.38), respectively; nor eczema; RR 1.06 (CI 0.73 – 1.55), RR 1.19 (CI 0.81 – 1.75) and RR 1.23 (CI 0.82 – 1.84), respectively.
There appeared to be a trend towards an increased risk of asthma for almost all types of URTI before the age of 2 years, between ages 2 and 4 years and between ages 4 and 8 years.
In a sensitivity analysis, in which different cut-off points were studied, the results did not change. We found no indication for an u-shape relation between recurrent URTI and asthma, allergic rhinitis and eczema.
Antibiotic treatment for URTI between ages 2 and 4 years was positively associated with adult asthma; RR 1.56 (CI 1.04 – 2.34); there was no association with allergic rhinitis and eczema.
Also, the relationship between having older siblings, a surrogate marker of childhood infections, and atopic disease at age 21 years showed a trend towards an increased risk of asthma and eczema; RR 1.30 (CI 0.86 –1.96) and RR 1.38 (CI 0.93 – 2.04), respectively.
In contrast, type B tympanograms between ages 2 and 4 years showed a trend towards a negative association with asthma; RR 0.71 (CI 0.39 – 1.30) and was negatively associated with allergic rhinitis; RR 0.72 (CI 0.52 – 0.98). This trend also remained after adjustment for day-care attendance.
Lower respiratory tract illness before age 2 years ($LRTI_{0-2}$) showed a positive trend, and between ages 2 and 4 years ($LRTI_{2-4}$) it was positively associated with asthma at age 21 years; RR 1.61 (CI 0.96 – 2.69) and RR 1.91 (CI 1.26 – 2.87). Similar relative risks were found if LRTI was defined as a doctor's visit because of pneumonia and/or dyspnoea and/or bronchitis but not asthma; RR 1.63 (CI 0.98 – 2.72) and RR 1.84 (CI 1.22 – 2.79), respectively.

Discussion

In this longitudinal study, following a birth cohort from early childhood into adulthood, defined recurrent childhood upper respiratory tract infections (URTI) did not decrease the risk of developing atopic disease later in life. This was true both for documented URTI, as well as for having older siblings as a surrogate marker of respiratory infections. If there is any association, the results rather suggest an increased risk of adult asthma in children having recurrent URTI during their childhood. Similarly, children having lower respiratory tract illness (LRTI) tended to have an increased risk of asthma in adulthood.

The relationship between respiratory tract infections in early life and subsequent development of asthma is still controversial. Long-term prospective studies with comprehensive documentation of URTI are scarce.
Several cross-sectional studies reported positive associations between early URTI and asthma in later childhood. However, in these reports, the association

between family size and atopic development was inverse.[10,11,19] As respiratory infections in children are usually mild and self-limiting, retrospective data collection might induce an underestimation of the number of infections experienced. Furthermore, it is likely that only the more severe infections are reported. Therefore, in cross-sectional studies recall bias might be a serious problem. In the present study, data on both URTI and LRTI were collected prospectively between ages 2 and 4 years. The 3-monthly visits of the children by trained nurses ensured a comprehensive and detailed data set on respiratory infections during an important period of childhood.

Similar to the present study, the Tucson Children's Respiratory Study (Tucson Study) and the German Multicentre Allergy Study (MAS) followed children prospectively during the first years of life.[20,21]

The Tucson Study investigated the relationship between paediatrician-confirmed LRTI in the first 3 years of life and asthma up to age 13 years.[20] Whereas LRTI were positively associated with subsequent asthma and hay fever, associations of family size and day care attendance with asthma were inverse. The MAS is the only prospective study so far with detailed prospective assessment of both URTI and LRTI during early childhood.[21] This birth cohort was followed up at age 1, 3, 6, 12 and 18 months and from then on at yearly intervals up to age 7 years. Children with repeated episodes of runny nose before the age of 1 year or having had a herpes virus infection in the first 3 years were less likely to develop asthma at the age of 7 years.

The differences of these two studies with the findings in the present study might be due to differences in study design, differences in definitions of early URTI and differences in length of follow up. The Tucson Study, like many other studies, used surrogate markers for respiratory infections, like number of siblings and day care attendance. Although it is reasonable to assume that frequent contacts with other children will result in an increased incidence of respiratory infections[8,9], the use of these surrogate markers potentially introduces some pitfalls. Other environmental influences and non-respiratory infections like hepatitis A and other gastrointestinal infections could bias these markers.[22] This might explain the above mentioned paradox of the positive association between URTI and atopic disease on one hand and the negative association between both day care and older siblings and atopic disease on the other hand. In our cohort, however, both frequent URTI and older siblings as surrogate marker of infectious burden pointed at the same direction, i.e. a positive trend.

Studies published so far also differed in their results regarding the effect of URTI and LRTI. Although the pathophysiology of the upper and lower airways is similar in many ways, differences between URTI and LRTI in their relationship with subsequent asthma have been observed. Like the MAS and the Tucson Study, we found a positive association between the occurrence of childhood LRTI and adult asthma. This suggests that congenital properties of the airways and the immune system predisposes children to lower airway disease both during childhood and adulthood. In this respect LRTI are probably not the cause of asthma, but rather the manifestation of the same predisposition. Recent longitudinal data on lung function in subjects with and without asthma support this hypothesis.[23,24] A genetic predisposition towards atopic disease may influence susceptibility to both URTI and LRTI as well. Our finding that children

of parents having atopic disease had more respiratory tract infections (data not shown) and a strong association between childhood recurrent URTI and LRTI (data not shown) supports this. This observation is confirmed by other studies.[8,25]
Another reason for conflicting results regarding the "hygiene hypothesis" might be differences in the definition of asthma. In the present study the outcomes of asthma, allergic rhinitis and eczema were defined according to symptoms experienced over the previous 12 months. Besides, subjects were followed until young adulthood, whereas studies following children up to the age of 8 to 10 years are probably concerning another phenotype of asthma since many adults with asthma have had no manifestations of this condition during childhood.[26]

To appreciate the results of this study some limitations should be discussed.
First, it is possible that information collected retrospectively over the first two years of life has not been accurate enough. It is often suggested that the Th1/Th2 balance of the immune system is influenced particularly by environmental factors in the first year of life. With respect to $URTI_{0-2}$ there may have been some misclassification, but other important variables like number of older siblings, being breastfed, maternal education and parental smoking have been measured correctly. Besides, the east-west German studies[27] showed that environment exposures in children aged 3 to 4 years also influenced atopic sensitisation and allergic rhinitis. This suggests that atopic development is not only restricted to exposures in infancy. Second, the respondents (64%) might not have been a representative sample of the total cohort. To evaluate this, we compared some baseline characteristics of participating and non-participating subjects. No important differences were found with respect to sex, being breastfed, family size, day care attendance, parental smoking habits, maternal educational level and most important, the number of URTI.
Third, in contrast to the other infection parameters, recurrent type B tympanograms tended to protect against asthma and allergic rhinitis in adulthood. The association though, was not very strong. Middle ear effusion often is closely related to URTI. Therefore, one would expect relative risk estimates similar to those for the other types of URTI. This finding may be coincidental.
Fourth, the power of our study might be a problem. With a baseline risk of 10%, 86 cases, and an alpha of 0.05, we were able to detect a difference of 20% with a power of 0.85. Smaller differences, which might be clinically relevant too, will never become statistically significant. It is, however, known that increasing the sample size only marginally influences the effect estimates. As our relative risks even suggest a positive association between recurrent URTI and asthma and eczema, a protective effect is not very likely.

In conclusion, in this birth cohort we did not find evidence to support the hypothesis that recurrent childhood URTI protect against atopic disease. The trend towards an increased risk of asthma in subjects who experienced recurrent childhood URTI and LRTI are in agreement with the hypothesis that genetically predisposed individuals have a greater risk of both respiratory tract infections and asthma.

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Chapter 3 B

Childhood respiratory infections, adult atopy and lung function: follow-up study up to age 21 years

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Childhood respiratory infections, adult atopy and lung function: follow-up study up to age 21 years

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Abstract

Study objectives
The hygiene hypothesis proposes a protective effect of childhood infections on atopic development. The role of childhood respiratory infections, however, remains controversial. We investigated the association between documented childhood upper respiratory tract infections and objective markers of atopy and asthma in adults.

Methods
A birth cohort of 1328 members was followed prospectively from ages 2 to 21 years. At ages 2 to 4 years detailed information on upper respiratory tract infections was collected every 3 months. At age 21 years lung function, exhaled bronchial nitric oxide (FeNO), total and specific IgE were measured.

Results
Of the original cohort, 404 members completed the investigation at age 21 years. Lung function (FEV_1 FVC and reversibility), IgE and Phadiatop were similar for subjects with and without recurrent upper respiratory tract infections in childhood. FEV_1/FVC ratio was slightly lower in participants who experienced recurrent upper respiratory tract infections before 2 years of age than in those who did not; mean 85.6 (SD 6.2) versus 87.3 (SD 6.1). The median FeNO level was slightly lower in those who experienced recurrent upper respiratory tract infections before 2 years of age than in those who did not; 17.3 (range 5 - 122) ppb versus 19.9 (range 6 - 155) ppb.

Conclusions
In young adults, recurrent upper respiratory tract infections experienced during childhood were not associated with atopy. Our findings do not support the hygiene hypothesis.

Introduction

The role of respiratory tract infections during childhood in the development of atopic disease and asthma later in life remains controversial; both positive[1-8] and negative[9 10] associations have been found.
Since Strachan's publication on the 'hygiene hypothesis' [10], many epidemiological studies have reported an inverse association between surrogate markers of childhood infections (e.g. family size or day-care attendance) and allergic rhinitis and asthma later in life, suggesting a protective effect of childhood infections.[11-14] When using surrogate markers of infectious burden it is, however, possible that non-respiratory infections and other environmental exposures are causal factors in the association. Prospective studies associating detailed information of upper respiratory tract infections (URTI) in the first years of life with allergic disease later in life are scarce[9] and lack follow up beyond childhood. We recently reported on the association of URTI and atopic disease using a questionnaire-based diagnoses[15 22], whereas it would also be interesting to study more objective measures like IgE, lung function and exhaled nitric oxide. We therefore investigated the association between childhood recurrent URTI and these objective markers in our birth cohort.

Methods

Study population and follow up
All children born between September 1982 and September 1983 in the city of Nijmegen, the Netherlands, were invited to participate in a study on otitis media at their second birthday (N=1439). Of this birth cohort 1328 subjects have been followed prospectively from ages 2 to 4 years; detailed parental reports on upper respiratory tract infections (URTI) during this period are available.[16 17]
At age 2 years a thorough history was taken of both URTI and lower respiratory tract illness (LRTI) during the first two years of life. Baseline parameters such as duration of pregnancy, birth weight, duration of breastfeeding, parental smoking, number of siblings and educational level of the parents were collected. Between ages 2 and 4 years children were visited at home every three months by a trained study nurse. At every three-monthly visit a detailed standardised questionnaire regarding URTI and lower respiratory tract illness (LRTI) in the previous three months was filled out.[16] At the age of 21 years participants were invited for assessment of the presence of atopic disease.[15] Via the City Council of Nijmegen the home addresses were traced and cohort members were invited to participate in lung function testing.
All subjects gave written informed consent to participate in the study. The study was approved by the ethical committee of the University Medical Centre Utrecht.

Recurrent childhood upper respiratory tract infections
Recurrent upper respiratory tract infections before the age of 2 years were defined as the occurrence of two or more ear infections (otalgia with fever and/or otorrhoea) and/or two or more throat infections over that period and/or at least five episodes of a runny nose in the previous twelve months.

Between ages 2 and 4 years the number of three-monthly episodes in which an upper respiratory tract infection had occurred was counted. Recurrent upper respiratory tract infections between ages 2 and 4 years were defined as at least three episodes of 3 months in which an ear infection had occurred (otalgia with fever and/or otorrhoea) and/or at least three episodes in which a throat infection had occurred and/or at least six episodes in which a runny nose had occurred.
The cut-off points were based on the median distribution of each type of respiratory tract infection at each assessment.

Childhood lower respiratory tract illness
Lower respiratory tract illness before the age of 2 years was defined as at least one visit to a physician over that period because of pneumonia and/or dyspnoea and/or asthma and/or bronchitis as reported by the parents.
Lower respiratory tract illness between ages 2 and 4 years was defined as a doctor's visit during any three-monthly episode because of pneumonia and/or dyspnoea and/or asthma and/or bronchitis as reported by the parents.

Lung function
Study members were asked to perform spirometry in a mobile lung function lab using the Lilly pneumotachometer system (Jaeger, Masterscreen, Hochberg, Germany). Subjects were asked not to use a short-acting bronchodilator 6 hours in advance and /or a long-acting bronchodilator 36 hours in advance.
Three technically acceptable flow volume curves and forced expiratory volume in one second (FEV_1) were obtained. The best curve was selected according to the criteria of the American Thoracic Society.[18] The best FEV_1 and FVC-values for each subject were selected and compared with gender- and height-related reference values (expressed as percentage of the predicted value) according to the European Community of Coal and Steel.[19]
Reversibility of bronchial obstruction was defined as the increase in the FEV_1, expressed as percentage of the predicted reference value (ΔFEV_1% predicted), measured after inhalation of 800 microgram of salbutamol (Ventolin Diskus®).

Fractional exhaled nitric oxide
Fractional exhaled bronchial nitric oxide (FeNO) measurements were performed with offline bag collection, formerly described by Pijnenburg et al.[20] and according to ATS criteria.[21] Subjects were asked to inhale through a nitric oxide filter in order to remove ambient NO. Subsequently they were asked to exhale with a constant target mouth pressure of 15 mbar. After five seconds of exhalation of dead space air, the (150 Ml) Mylar bag was filled during another 5 seconds. After a resting period of at least 30 seconds the second bag was filled, following the same procedure. Within eight hours the collected air samples were analysed using the NIOX® system (Aerocrine AB, Stockholm, Sweden). Two air samples were taken from each bag. Out of the four values a mean FeNO value was calculated, expressed as parts per billion (ppb). Calibration was performed routinely.

Atopy
Blood sampling was performed by vena puncture and serum samples stored at -20 C until analysis. Total IgE and specific IgE was determined by a solid phase immunometric assay on an ImmunoCAP 250 system of Pharmacia (Pharmacia Diagnostics AB, Upsala, Sweden).
Specific IgE against a mixture of 10 common aeroallergens, like birch, timothy, cat, dog, horse and *Dermatophagoides pteronyssinus* was determined by Phadiatop, which measures IgE antibodies in serum. Results were expressed as positive (atopic) or negative (non-atopic).

Statistical analysis
T-tests, Mann-Whitney tests, and chi-square tests were used to evaluate possible differences in means, medians, and percentages between children with and without URTI and LRTI. ANCOVA and Mantel Haenzel procedures were used to adjust for potential confounding (e.g. passive smoking, and atopic parents). Since the effect estimates were not influenced by these adjustments, crude effect estimates are presented. All analyses were performed with SAS version 8.0.

Results

Addresses of 1055 subjects (79%) of the 1328 original cohort members could be traced; 404 of them agreed to participate in the follow-up at age 21 years. Baseline characteristics of the original cohort and participants are shown in Table 3.3.

Prospectively documented recurrent URTI between ages 2 to 4 years and retrospectively documented recurrent URTI before 2 years of age were not associated with median total IgE and Phadiatop (Table 3.4).

Recurrent URTI between ages 2 to 4 years and recurrent URTI before 2 years of age were not associated with lung function parameters at age 21 years (Table 3.4). The mean FEV_1 and FVC were similar for participants who experienced recurrent childhood URTI and those who did not. In addition, there was no difference in median reversibility of bronchial obstruction between the two groups. Only for the FEV_1/FVC ratio was a small, but significant, difference found between those who did and those who did not experience URTI between the ages 0 to 2 years; 85.6 (SD 6.2) versus 87.3 (SD 6.1), p=0.02.
Median FeNO was slightly lower in participants who did versus those who did not experience URTI at ages 0 to 2 years; 17.3 (range 5 - 122) ppb versus 19.9 (range 6 - 155) ppb, (p=0.01) (Table 3.4).

Table 3.3 Characteristics of the current study population compared with the original cohort.

	Study population N=406 N (%)	**Original cohort N=1328** N (%)
Male gender	155 (38)	666 (50)
Breast fed > 3 months	138 (36)	402 (33)
Older siblings	228 (57)	652 (54)
Smoking mother	175 (44)	539 (42)
Educational level mother		
low	138 (34)	597 (45)
medial	166 (41)	439 (33)
high	102 (25)	292 (22)
Recurrent URTI*		
age 0-2 years	173 (44)	550 (44)
age 2-4 years	193 (50)	531 (44)
LRTI†		
age 0-2 years	51 (13)	182 (14)
age 2-4 years	92 (23)	261 (20)

*URTI is upper respiratory tract infections; †LRTI is lower respiratory tract illness

A slightly lower lung function (expressed as FEV_1/FVC ratio) was found in participants who experienced LRTI at ages 0 to 2 years and 2 to 4 years versus those who did not; mean FEV_1/FVC ratio 84.5 (SD 7.6) versus 86.9 (SD 6.3) (p=0.04) and 85.1 (SD 7.4) versus 86.9 (SD 6.3) (p=0.04), respectively (Table 3.4). LRTI before the age of 2 years and between ages 2 to 4 years were not associated with FEV_1, reversibility of bronchial obstruction, IgE, Phadiatop and FeNO values at age 21 years.

Discussion

Principal findings

In this longitudinal study following a birth cohort from early childhood to adulthood, documented recurrent URTI in childhood were not associated with objective markers of allergic sensitization (IgE and Phadiatop) and lung function (FEV_1, FVC and reversibility of bronchial obstruction) later in life. These findings are in agreement with our earlier publication in which we could not find a protective effect of childhood URTI on questionnaire-based diagnosis of asthma and atopic disease.[15 22]

Table 3.4 Means (SD) and medians (range) of lung function parameters,IgE, Phadiatop and FeNO in subjects who experienced recurrent upper respiratory tract infections (URTI yes) versus subjects who did not (URTI no) and in those who experienced lower respiratory tract illness (LRTI yes) versus those who did not (LRTI no) between 0 to 2 and 2 to 4 years of age

		FEV_1* %pred mean	p-value	**FVC†** %pred mean	p-value	**FEV_1/FVC§** ratio l/l mean	p-value		
URTI 0-2y	**yes**	116.3 (13.6)		113.8 (12.5)		85.6 (6.2)			
	no	116.3 (14.5)	0.99	112.0 (13.4)	0.18	87.3 (6.1)	0.02		
URTI 2-4y	**yes**	116.5 (13.7)		112.8 (12.1)		87.6 (5.6)			
	no	115.4 (14.7)	0.43	112.2 (13.8)	0.69	87.5 (6.2)	0.81		
LRTI 0-2y	**yes**	115.3 (12.4)		114.3 (11.5)		84.5 (7.6)			
	No	117.9 (14.3)	0.61	112.6 (13.2)	0.38	86.9 (6.3)	0.04		
LRTI 2-4y	**yes**	116.0 (13.3)		114.1 (11.8)		85.1 (7.4)			
	no	116.0 (14.6)	0.98	112.2 (13.4)	0.22	86.9 (6.3)	0.04		
		Total IgE kU/l median	p-value	**Phadiatop** positive %	p-value	**FeNO‡** ppb median	p-value	**Reversibility#** ΔFEV_1 %pred median	p-value
URTI 0-2y	**yes**	61.2 (3- 5000)		43		17.0 (5.1- 59.2)		5.0 (6 - 18)	
	no	52.4 (0- 5000)	0.69	43	0.99	19.5 (6.2- 85.1)	0.03	4.0 (10 - 28)	0.22
				45		19.1 (7.2- 85.1)		4.0 (10 - 21)	
URTI 2-4y	**yes**	60.7 (0- 5000)	0.62	41	0.45	18.2 (5.1- 79.8)	0.57	4.0 (7 - 28)	0.39
	no	54.4 (0- 5000)							
LRTI 0-2y	**yes**	63.9 (5- 2965)		39		20.6 (8.0- 85.1)		5.0 (3 - 20)	
	no	54.1 (0- 5000)	0.44	44	0.52	18.1 (5.1- 79.8)	0.62	4.0 (10 - 28)	0.59
LRTI 2-4y	**yes**	48.8 (2- 5000)		43		19.8 (7.6- 85.1)		4.0 (5 - 18)	
	no	58.4 (0- 5000)	0.91	43	0.92	18.5 (5.1- 79.8)	0.69	4.0 (10 - 28)	0.21

URTI is upper respiratory tract infections; LRTI is lower respiratory tract illness

*FEV_1 %pred is forced expiratory volume in 1 second, expressed as percentage of the predicted reference value; †FVC %pred is forced vital capacity, expressed as percentage of the predicted value; §FEV_1/FVC ratio is FEV_1 in litres divided by the forced vital capacity in litres; ‡FeNO is exhaled nitric oxide expressed in parts per billion (ppb); #Reversibility is the change in FEV_1, as percentage of predicted (ΔFEV_1 %pred), after bronchodilation with 800 microgram salbutamol.

404 subjects performed the lung function tests; blood samples were obtained from 395 participants; 361 subjects performed FeNO measurements.

Strength of study

Until now, very few prospective birth cohort studies on early childhood infections and development of atopic diseases have been published[2 4-6 8 9] and many of them are based on retrospective and cross-sectional data or primary health care records.[1 3 7] Because recall bias is a considerable problem in a generally self-limiting condition like URTI, associations found in these studies should be interpreted with caution. The few studies including detailed and comprehensive data on URTI in early childhood lack follow-up beyond childhood.[6 8 9] In the present study, data on upper and lower respiratory tract infections were collected prospectively at 3-monthly evaluations between the ages 2 and 4 years and therefore reflect the true incidence of symptomatic respiratory infections.[15 16] This is also the first study to assess a combination of objective markers for both asthma and atopy and a follow-up up to age 21 years.

Total IgE and specific IgE were the same in adults who experienced early recurrent URTI and in those who did not. Our results do not support the assumption that common colds are of importance in the development of atopic sensitisation. This is in agreement with recent results from the Oslo Birth cohort in which no protective effect was seen from early childhood URTI on skin prick sensitisation at the age of 10 years.[8] Another study described a decreased total IgE at age 14-15 years in study members experiencing gastrointestinal and respiratory infections in the first 6 months after birth.[23] We could not confirm the latter finding; however, the study did not assess specific IgE and the investigated children were only a small selected sample of the total cohort, which might influence generalizability of the results.

Furthermore, no consistent associations were found between early recurrent URTI and lung function later in life. The only difference found was a slightly lower FEV_1/FVC ratio in adults who had experienced recurrent URTI before 2 years of age. A similar association was found between childhood LRTI and FEV_1/FVC ratio in adults. The association of LRTI in childhood and decreased lung function in adulthood might result from airway remodelling. Another hypothesis is the concept of reversed causation, which proposes that children with impaired lung function are more prone to develop lower respiratory tract infections during childhood. This concept is in line with the results of the Tucson Respiratory Studies, showing that lower lung function shortly after birth is associated with a higher risk of childhood wheezing LRTI.[24] Lung function of the early wheezers was lower before the first wheezing period and remained lower up to age 16 years, compared to never and late onset wheezers.[25]

FeNO was decreased in participants experiencing recurrent URTI before the age of 2 years. Many studies showed that increased FeNO can be considered as a marker of atopy or eosinophilic inflammation.[26 27] Though we could not find any association with atopic sensitization this result might prove a protective effect of URTI on allergic airway inflammation later in life.

Potential weakness of study

Some limitations of the present study should be discussed.

First, the subjects who participated in the follow-up at adulthood (N=404) might not be representative for the entire birth cohort (N=1328). However, no important differences were found in baseline characteristics between participating and non-participating subjects (Table 3.3). The only difference

found between the two groups was the number of participating females, for which we have no explanation. Nonetheless, we can not completely rule out a positive selection of atopic or asthmatic candidates, or candidates who are still suffering from recurrent respiratory tract infections who might have been more motivated to participate. Such a selection, however, would have resulted in an overestimation of the effect.
Second, data on URTI in the first year were gathered retrospectively at the age of 2 years. It is often suggested that environmental exposures especially in the first year of life influences atopic development, whereas we focussed on childhood URTI between ages 2-4 years. The east-west German studies[28] have shown that also changing environmental exposures after infancy in preschool children resulted in increasing prevalences of atopic sensitization and allergic rhinitis, possibly due to improving living standards. We could, however, not confirm such an association between childhood exposure to recurrent URTI and atopic sensitisation in adulthood.
Third, similar to previous studies, we studied associations with symptomatic respiratory infections, based on parental reports. Asymptomatic or very mild infections in early life, although potentially of importance in the development of the immune system and thus protecting individuals against developing atopic disease, have not been included in this way.

General conclusion
In conclusion, in this birth cohort we did not find evidence to support the hypothesis that recurrent childhood URTI may protect against atopic development later in life.

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Chapter 3 C

Adenoidectomy and/or Tonsillectomy in childhood is not associated with atopic disease later in life

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Adenoidectomy and/or tonsillectomy in childhood is not associated with atopic disease later in life

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Abstract

Objective
To investigate the association between adenoidectomy and/or tonsillectomy in childhood and asthma, allergic rhinitis (AR), and eczema in adolescence.

Methods
Longitudinal birth cohort study of 1328 members born in the city of Nijmegen. Information on ear–nose–throat surgery was documented at 2, 4, and 8 years of age. In 1055 cohort members the incidence of asthma, AR, and eczema at 21 years of age was determined using the International Study of Asthma and Allergic disease in Childhood Core Questionnaire. To analyse the association between adenoidectomy and/or tonsillectomy in childhood and asthma, AR, and eczema at age 21 years, relative risks (RR) were calculated.

Results
Six hundred and ninety-three (66%) members completed the questionnaire at age 21 years, of whom 104 (15%) had undergone adenoidectomy and/or tonsillectomy and 262 (38%) reported atopic disease. Children who underwent adenoidectomy and/or tonsillectomy before the age of 8 years were not more likely to develop asthma, AR, or eczema at the age of 21 years than children who did not; RR 0.93 (95% confidence limits (CL) 0.52–1.64), RR 0.94 (CL 0.68–1.30), and RR 1.00 (CL 0.59–1.68), respectively.

Conclusions
Our data show no association between adenoidectomy and/or tonsillectomy in childhood and the incidence of atopic disease in young adults.

Introduction

The prevalence of asthma and other atopic diseases like allergic rhinitis and eczema is increasing, especially in Western societies.[1,2] Both genetic predisposition and environmental factors play a role in the pathogenesis of atopy.[3] In 1989, Strachan hypothesized that an improved hygienic lifestyle enhances the risk of the development of atopic diseases, the so-called 'hygiene hypothesis'.[4] Exposure to allergens and viral or bacterial infections during childhood is proposed to skew the immune system from a T-helper type 2 (Th2) cell into a T-helper type 1 (Th1) cell regulated immune response.[5,6] It has been speculated that disturbances of the immune system, particularly in the Th1–Th2 balance, play an important role in the development of atopic diseases.[7-9]
Adenoidectomy and tonsillectomy are common surgical procedures in young children with recurrent upper respiratory tract infections.[10,11] Only scarce data are available on their long-term effects. Tonsils and adenoids are part of Waldeyer's ring and as such involved in the defence against airborne and alimentary micro organisms.[12,13] Therefore, one might speculate that surgical removal of adenoid and/or tonsil tissue results in reduced humoral and cellular responses and thus reduces stimulation of the immune system in young children.[14] In line with the 'hygiene hypothesis' this decrease of stimulation might result in an increasing risk of atopic disease in later life. We therefore tested the hypothesis that adenoidectomy and tonsillectomy at an early age are associated with an increased risk of asthma, allergic rhinitis, and eczema at adolescence in a large prospective birth cohort study followed from ages 2 to 21 years.

Methods

Study population and ear–nose–throat interventions
A birth cohort of 1328 Dutch children born between 1 September 1982, and 31 August 1983, living Nijmegen (the Netherlands) has been followed prospectively from ages 2 to 8 years.
At 2 years of age, baseline characteristics such as duration of pregnancy, birth weight, breastfeeding, parental smoking, number of siblings, day care attendance, and educational level of the parents were collected. All ear–nose–throat (ENT) interventions including adenoidectomy and tonsillectomy during the first 2 years were recorded. Between 2 and 4 years of age, all children were followed prospectively by 3-monthly interviews regarding ENT interventions. At the age of 8 years the children were re-evaluated by means of a standardized questionnaire regarding ENT interventions that occurred between the ages of 4 and 8 years.[15]

Atopic disease at age 21 years
At the age of 21 years all cohort members were invited for re-assessment; via the City Council of Nijmegen, their home addresses were traced. They were sent the International Study of Asthma and Allergic disease in Childhood (ISAAC) Core Questionnaire, which has been validated to obtain data on history

asthma, allergic rhinitis, and eczema.[16] Furthermore, information on parental of atopic diseases was obtained. The latter was defined as asthma, allergic rhinitis, or eczema present in at least one of the parents.
The study was approved by the medical ethics committee of the University Medical Centre Utrecht.

Asthma was defined as the occurrence of wheezing in the last 12 months and/or a doctor's diagnosis of asthma in that same period and/or current use of asthma medication.
Allergic rhinitis was defined as the presence of a runny or blocked nose without having a cold or flu in the last 12 months, accompanied by itchy-watery eyes and/or some interference of this nose problem with daily activities.
Eczema was defined as itchy skin rash that has been coming and going for at least 6 months and affecting particular skin sites (i.e. folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes) in the last 12 months.

Statistical analysis
We estimated the relative risks to analyse the association between adenoidectomy and/or tonsillectomy in childhood on asthma, allergic rhinitis, and eczema at age 21 years. Mantel–Haenszel analyses were performed to adjust for potential confounders as well as to study effect modifiers, e.g. parental smoking, parental history of atopy, parental educational level, breastfeeding, birth weight, and gestational age. The final rate ratios were adjusted for maternal smoking and parental history of atopy as these factors were indeed found to be confounders.
SAS software (version 8.0) was used for all statistical analyses.

Results

Response
Addresses of 1055 subjects (79%) of the 1328 original cohort members could be traced; 693 subjects (66%) responded by returning the questionnaire.

Baseline characteristics and ear–nose–throat interventions
Of the 693 respondents, 374 (54%) were females, 299 (44%) had a mother who smoked, and 315 (46%) had a parental history of atopy (Table 3.5).
A total of 104 participants (15%) had undergone adenoidectomy and/or tonsillectomy before the age of 8 years (Table 3.5).
Responding and non-responding cohort members were comparable according to the baseline characteristics and ENT interventions.

Outcome measures
At age 21 years, 86 (12%) of the 693 subjects who participated in the follow-up had asthma according to our definition; 214 subjects (31%) met the criteria for allergic rhinitis and 98 (14%) for eczema. A total of 262 (38%) subjects had at least one of the atopic diseases.

Table 3.5 Characteristics of the study population (693 responders)

	N (%)
Female gender	374 (54)
Family size:	
any sib	647 (94)
more than 1 sib	378 (55)
Breast fed more than 3 months	222 (34)
Smoking mother	299 (44)
Educational level mother:	
low	278 (40)
medium	257 (37)
high	158 (23)
Parental atopy of at least 1 parent	315 (46)
Adenoidectomy and/or tonsillectomy:	
0 – 2 years	10 (1)
2 – 4 years	50 (7)
4 – 8 years	72 (50)
0 – 8 years	104 (15)
Atopic disease at age 21 years:	
Asthma	86 (12)
Allergic rhinitis	214 (31)
Eczema	98 (14)
At least one atopic disease	262 (38)

Table 3.6 Relative risks (95% confidence limits (CL)) of asthma, allergic rhinitis and eczema in 693 subjects at 21 years who had undergone adenoidectomy and/or tonsillectomy in childhood

	Asthma (95% CL)	**Allergic rhinitis** (95% CL)	**Eczema** (95% CL)
Adenoidectomy and/or tonsillectomy			
0 – 2 years	0.78 (0.12 – 5.21)	0.97 (0.31 – 2.58)	1.24 (0.35 - 4.44)
2 – 4 years	0.94 (0.43 – 2.04)	1.03 (0.68 – 1.58)	1.23 (0.64 - 2.38)
4 – 8 years	0.90 (0.46 – 1.78)	1.03 (0.71 – 1.48)	1.05 (0.58 - 1.89)
0 – 4 years	0.93 (0.45 – 1.92)	1.05 (0.71 – 1.56)	1.03 (0.53 - 2.01)
0 – 8 years	0.93 (0.52 – 1.54)	0.94 (0.68 – 1.30)	1.00 (0.59 - 1.69)

Table 3.6 shows the relative risks (RR) of asthma, allergic rhinitis, and eczema at age 21 years adjusted for confounding factors, i.e. maternal smoking and parental history of atopy.

There was no significant association between adenoidectomy and/or tonsillectomy and asthma, allergic rhinitis, or eczema: RR 0.93 (95% confidence limits (CL) 0.52–1.64), RR 0.94 (CL 0.68–1.30) and RR 1.00 (CL 0.59–1.68), respectively. The age at which surgery was performed did not affect this association. (Table 3.6)

Discussion

In the present study, documented ENT interventions until the age of 8 years were compared with the incidence of asthma, allergic rhinitis, and eczema at 21 years of age in 693 participants. Adenoidectomy and tonsillectomy performed in childhood are not associated with atopic disease in later life. This contradicts our hypothesis that removing immunological competent tissue at young age increases the risk of developing atopic disease.

The ring of Waldeyer is an important part of the general immune system. Several studies showed slightly decreased serum Ig levels after adenoidectomy and tonsillectomy. Cantani et al. studied 65 children aged 2–11 years with normal levels of serum IgA, IgM, and IgG before surgery.[17] At 1 and 4 months after tonsillectomy, serum Igs were significantly lower. Jeschke and Ströder observed a cohort of 106 children who underwent tonsillectomy at the age of 3–15 years; serum IgG, IgA, and IgM and secretory IgA gradually fell during follow-up to 38 months.[18] Other studies reported the same changes in Ig levels after adenoidectomy and tonsillectomy compared with a control group.[19-21] Beside changes in humoral immune responses, changes in the cellular immune system have also been described after adenoidectomy and tonsillectomy. Several authors reported changes in B and T cells, as well as in different T cell subsets.[20,22,23] The short-term effects on humoral and cellular immune responses might argue for a more reserved attitude towards performing adenoidectomy and tonsillectomy in children. Although one can speculate that short-term changes might proceed into immunological imbalance on the longrun, long-term data are lacking. In view of these immunological observations and the 'hygiene hypothesis' we studied the long-term clinical consequences of adenoidectomy and tonsillectomy in childhood on the development of atopic disease.

Our findings are in contrast with those from the British National Child Developmental Study (NCDS).[24] In this latter national cohort study, children who had adenoidectomy or tonsillectomy before the age of 7 years had a 1.4-fold risk of developing asthma at age 12–16 years. Differences in asthma phenotypes at different ages might explain the difference between both studies. Moreover, the association that was found in the NCDS was based on univariate analysis, and lack of potential confounding factors might also explain part of the discrepancy. Associations with other atopic diseases, such as allergic rhinitis and eczema, were not studied in the NCDS.

In our cohort, we had the unique opportunity to study the effects of adenoidectomy and tonsillectomy over a prolonged period of time. The large birth cohort provided an excellent basis to study interaction without introduction of selection bias. A potential limitation of our study is the use of a questionnaire

to define the outcome parameters of atopic disease. A more objective measure for atopic disease, like pulmonary function testing, might have improved the validity of the outcome. The ISAAC questionnaire has, however, been validated in many settings.[16]
Another potential limitation is the fact that only 693 (52%) of the original 1328 study members could be evaluated in the final analysis. This might introduce selection bias as study members with atopic disease could have been more motivated to participate. The most important reason for loss to follow-up, however, was that study members could not be traced because they had moved. Moreover, a comparison of the baseline characteristics of the study members with complete data and the original cohort showed no differences between both groups. We therefore, believe that selection bias can be considered minimal.
The debate about performing adenoidectomy and tonsillectomy is still ongoing. A recent study by van Staaij et al. showed that children having repetitive upper respiratory tract infections find relief within the first 6 months following adenoidectomy and/or tonsillectomy.[25] However, in comparison with the so-called watchful waiting treatment all differences between the two groups had disappeared by the period of 24 months. In addition to the question about long-term adverse effects, long-term effectiveness should be weighted in the indication for adenoidectomy and tonsillectomy in children. Our data do not support the idea that adenoidectomy and/or tonsillectomy in childhood is harmful in terms of atopic development, but in the absence of noticeable long-term benefits, a more conservative attitude is indicated.

Conclusions
No relationship has been found between adenoidectomy and tonsillectomy in childhood and the incidence of atopic diseases at adolescence.

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Chapter 4

A prediction rule of asthma in young adults was developed using childhood characteristics

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A prediction rule of asthma in young adultes was developed using childhood characteristics

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Abstract

Objective

To develop an easily applicable prediction rule for asthma in young adulthood using childhood characteristics.

Methods

A total of 1055 out of 1328 members of a Dutch birth cohort were followed from 2 to 21 years of age. Univariate and multivariate logistic regression analyses were used to evaluate the predictive value of childhood characteristics on asthma at 21 years of age. A prognostic function was developed, and the area under the receiving operating characteristic (ROC) curve was used to estimate the predictive ability of the prognostic models.

Results

Of the 693 responding subjects, 86 (12%) were diagnosed with asthma. Independent prognostic factors at ages 2 and 4 years were female gender (odds ratios (OR) 1.9 and 2.1; 95% confidence intervals (CI) 1.2 - 3.2 and 1.3 – 2.5), smoking mother (OR 1.6 and 1.6; CI 1.0 – 2.7 and 1.0 – 2.6), lower respiratory tract illness (OR 1.9 and 2.4; CI 1.0 – 3.6 and 1.4 – 4.0) and atopic parents (OR 2.1 and 1.9; CI 1.3 – 3.4 and 1.2 – 3.1). The predictive power of both models was poor; area under ROC curve was 0.66 and 0.68, respectively.

Conclusion

Asthma in young adulthood could not be predicted satisfactorily based on childhood characteristics. Nevertheless, we propose that this method is further tested as a tool to predict development of asthma.

Introduction

Asthma is an increasing health problem in Western societies.[1] Its pressure on health care facilities and budget is considerable. It is a challenge for primary health care to find ways to prevent and treat asthma, particularly in high risk patients, who will benefit most from interventions. Early treatment of asthmatic symptoms might for instance prevent airway remodeling.[2] Adequate risk assessment, preferably by using easily obtainable information, is however, still difficult to achieve. Once it is possible to assess the individual risk of young children in the general population, both preventive and therapeutic intervention strategies as well as proper prognostic information to parents can be targeted at this group.

Prediction models, that estimate the probability of occurrence of a relevant outcome as a combined function of the levels of various predictors, are a helpful tool in the assessment of individual prognosis.[3,4] Such clinical prediction models would be useful to distinguish individual children at high risk of developing asthma during adulthood from those at low risk.

So far, previous studies on this topic have focussed on single childhood risk factors associated with persistence or recurrence of asthma in adulthood, such as severe asthma in childhood, other atopic disease, parental asthma, female sex, active cigarette smoking, positive skin prick test, decreased lung function and bronchial hyper-responsiveness.[5-8] Although these studies show that the presence of such factors will increase the relative risk for developing asthma in adulthood, they do not enable us to quantify an absolute risk score for an individual subject. Only very few studies attempted to provide an individual risk assessment of asthma or persistence of wheezing in childhood.[9,10]

So far, only Toelle et al. have reported on a risk score to assess individual prognoses in adulthood based on data from the Belmont cohort study.[11] The authors described a diagnostic algorithm of independent likelihood ratios. However, they did not develop a clinical prediction rule. The aim of the present study is to define prognostic factors and to develop an easily applicable prediction model that can be used in a primary health care setting to identify children at risk for asthma in young adulthood.

Methods

Study population and follow up

All children born in the city of Nijmegen between September 1982 and September 1983 , were invited to participate in a study on otitis media at their second birthday (N=1439). Of this birth cohort, subjects have been followed prospectively from 2 to 4 years (N=1328).12,13

At age 2 years a thorough history was taken of both upper respiratory tract infections (URTI) and lower respiratory tract illness (LRTI) during the first two years of life. Baseline parameters such as duration of pregnancy, birth weight, duration of breastfeeding, parental smoking, number of siblings, day care attendance and educational level of the parents were collected. Between ages 2 and 4 years, children were visited at home every three months by a trained study nurse. At every three-monthly visit a detailed standardised interview regarding URTI and LRTI in the previous three months was taken.12 At the age

of 21 years participants were invited for assessment of the presence of atopic disease. Via the City Council of Nijmegen the home addresses were traced and cohort members were sent another standardised questionnaire.14
The study was approved by the local ethical committee of the University Medical Centre Utrecht.

Outcome
The Core Questionnaire of the International Study of Asthma and Allergy in Children (ISAAC) was used which has been validated to obtain data on asthma.14 Furthermore, we obtained data about current use of asthma medication.
Asthma at the age of 21 years was defined as the occurrence of wheezing in the last twelve months and/or a doctor's diagnosis of asthma in that same period and/or current use of asthma medication.

Potential predictor variables
We considered gender, older siblings, day care attendance, passive smoking, breastfeeding, premature birth, low birth weight, and educational level of parents, parental atopic disease, recurrent upper respiratory tract infections, lower respiratory tract illness, antibiotic treatment and ear, nose and throat (ENT) surgery as potential predictors of asthma at 21 years of age.
Parental history of atopic diseases was defined as asthma, allergic rhinitis or eczema in at least one parent.
Lower respiratory tract illness before the age of 2 years (LRTI0-2) was defined as at least one doctor's visit over that period because of pneumonia and/or dyspnoea and/or asthma and/or chronic bronchitis as reported by the parents at 2 years of age.
Lower respiratory tract illness between ages 2 and 4 years (LRTI2-4) was defined as a doctor's visit during any three-monthly episode because of pneumonia and/or dyspnoea and/or asthma and/or chronic bronchitis as reported by the parents at the three-monthly visits.
Recurrent upper respiratory tract infections before the age of 2 years (URTI0-2) were defined as the occurrence of two or more ear infections (otalgia with fever and/or otorrhoea) and/or two or more throat infections over that period and/or at least five episodes of a runny nose in the last twelve months as reported by the parents at 2 years of age.
Between ages 2 and 4 years the number of three-monthly episodes in which an upper respiratory tract infection had occurred was counted. Recurrent upper respiratory tract infections between ages 2 and 4 years (URTI2-4) were defined as at least three episodes in which an ear infection had occurred (otalgia with fever and/or otorrhoea) and/or at least three episodes in which a throat infection had occurred and/or at least six episodes in which a runny nose had occurred as reported by the parents at the three-monthly visits.
The cut-off points for recurrent URTI were based on the median distribution of these infections at each assessment.
Antibiotic treatment for URTI0-2 and URTI2-4 was defined as at least one antibiotic course, reported by the parents, for an upper respiratory tract infection in the corresponding period.

Statistical analysis
The association between each prognostic factor and the presence or absence of asthma in young adulthood was studied by univariate logistic regression analysis both at 2 and 4 years of age.
Prognostic factors univariately associated with outcome (P<.10) were included in a multivariate logistic regression model. The prognostic accuracy of the model was estimated by their reliability (goodness-of-fit) using Hosmer-Lemeshow statistics.15 The prognostic ability of the model, i.e. the power to discriminate between subjects with and without asthma, was estimated by measuring the area under the receiver operating characteristic (ROC) curve.16,17 The ROC curve is a plot of the true positive rate (sensitivity) versus the false positive rate (1 – specificity) evaluated at consecutive cut-off points of the predicted probability. The area under the ROC curve (AUC) provides a quantitative summary of the discriminative ability of a predictive model. A useless predictive model, such as a coin flip, would yield an AUC of 0.5. An AUC of 1.0 indicates that the model discriminates perfectly between subjects with and without asthma in young adulthood. We considered a value over 0.8 as adequate discriminative ability.
The final models at both 2 and 4 years of age were transformed into a clinical prediction rule. Regression coefficients were multiplied by 10, and rounded of to the nearest integer. Points were assigned to each variable and by adding up these results, a score was obtained for each subject. To simplify the interpretation of the model, various cut-off points were chosen to show the number of children developing or not developing asthma in young adulthood.

Results

At the age of 21 years, addresses of 1055 subjects (79%) of the 1328 original cohort members could be traced; 693 (66%) of these 1055 subjects returned the questionnaire.
The 693 study members evaluated at age 21 years and the original cohort of 1328 members did not differ significantly regarding sex distribution, history of breast feeding, family size, day care attendance, parental smoking habits, maternal educational level, recurrent upper respiratory tract infections, lower respiratory tract illness, and antibiotic treatment at age 2 years. (Table 4.1).
At age 21 years, 86 (12%) of the 693 subjects who participated in the follow up had asthma according to our definition.

Table 4.2 shows the absolute risks of asthma according to the known childhood factors. For example, a child with atopic parents had an absolute risk to develop asthma of 16%, whereas a child without atopic parents had a risk of 9%. In the univariate analyses female gender, smoking mother, atopic parents, asthmatic parents, recurrent URTI0-2 , recurrent URTI2-4 , LRTI0-2, LRTI2-4, antibiotic treat- ment for URTI0-2, and for URTI2-4, were associated with the occurrence of asthma at age 21 years (Table 4.2).
Inclusion of these determinants into multivariable logistic regression models and reduction to a model with the smallest number of factors without loosing much predictive power, led to the following results:

Table 4.1 Characteristics of the study population (N=693) as compared to the birth cohort (N=1328)

Characteristic	Prevalence among study members (N=693)	Prevalence in birth cohort (N=1328)
	% (number of study members)	
Male sex	46.0 (319)	50.2 (666)
Older siblings	45.3 (294)	46.3 (563)
Day care attendance	70.3 (428)	66.8 (744)
Passive smoking (mother)	44.2 (299)	42.1 (539)
Breast fed	69.9 (453)	69.1 (836)
Birth weight < 2500 g	8.8 (53)	8.8 (96)
Low educational level mother	40.1 (278)	44.9 (597)
Recurrent $URTI_{0-2}$	21.4 (148)	20.6 (274)
Recurrent $URTI_{2-4}$	31.1 (201)	28.4 (345)
$LRTI_{0-2}$	12.1 (84)	13.7 (182)
$LRTI_{2-4}$	13.0 (90)	12.4 (164)
Antibiotic treatment for $URTI_{0-2}$	21.4 (148)	20.6 (274)
Antibiotic treatment for $URTI_{2-4}$	41.6 (269)	39.3 (477)
ENT-$surgery_{0-2}$	1.9 (13)	1.9 (25)
ENT-$surgery_{2-4}$	19.9 (129)	18.2 (221)

Recurrent upper respiratory tract infections between ages 0 to 2 years ($URTI_{0-2}$) and 2 to 4 years ($URTI_{2-4}$); Lower respiratory tract illness between ages 0 to 2 years ($LRTI_{0-2}$) and 2 to 4 years ($LRTI_{2-4}$); Ear-nose-and throat-surgery between 0 to 2 years (ENT_{0-2}) and 2 to 4 years (ENT_{2-4})

Table 4.2 Absolute risks of asthma and univariate analysis of potential predicting factors for asthma at 21 years of age in 693 subjects

	Asthma N (%)	OR (95% CI)
Gender		
Girls	58 (16)	
Boys	28 (9)	2.0 (1.3 - 3.3)
Being breast fed		
Yes	59 (13)	
No	22 (11)	1.2 (0.7 - 2.0)
Duration breast feeding		
> 3 months	27 (12)	
≤ 3 months	54 (13)	1.0 (0.6 – 1.6)
Older siblings		
Yes	52 (14)	
No	34 (11)	1.3 (0.8 - 2.0)

Table 4.2 continued

	Asthma N (%)	OR (95% CI)
Day care attendance		
Yes	49 (11)	
No	26 (14)	0.8 (0.5 - 1.3)
Birth weight		
< 2500 g	8 (15)	
≥ 2500 g	66 (12)	1.3 (0.6 - 2.9)
Smoking mother		
Yes	46 (15)	
No	38 (10)	1.6 (1.0 - 2.6)
Educational level mother		
Low	39 (14)	
High	47 (11)	1.3 (0.8 - 2.0)
Atopic parent(s)		
Yes	51 (16)	
No	34 (9)	1.9 (1.2 - 3.1)
Asthmatic parent(s)		
Yes	23 (22)	
No	63 (11)	2.3 (1.3 - 4.0)
Recurrent $URTI_{0-2}$		
Yes	38 (13)	
No	45 (12)	1.0 (0.7 - 1.7)
Recurrent $URTI_{2-4}$		
Yes	48 (15)	
No	33 (10)	1.6 (1.0 - 2.6)
$LRTI_{0-2}$		
Yes	16 (19)	
No	67 (12)	1.8 (1.0 - 3.3)
$LRTI_{2-4}$		
Yes	29 (20)	
No	57 (10)	2.1 (1.3 - 3.5)
Antibiotics for $URTI_{0-2}$		
Yes	25 (17)	
No	61 (11)	1.6 (1.0 - 2.7)
Antibiotics for $URTI_{2-4}$		
Yes	44 (17)	
No	37 (10)	1.8 (1.1 - 2.9)
$ENT\text{-}surgery_{0-2}$		
Yes	2 (11)	
No	84 (13)	0.8 (0.2 - 3.6)
$ENT\text{-}surgery_{2-4}$		
Yes	12 (9)	
No	69 (13)	0.7 (0.4 - 1.3)

Recurrent upper respiratory tract infections between ages 0 to 2 years ($URTI_{0-2}$) and 2 to 4 years ($URTI_{2-4}$); Lower respiratory tract illness between ages 0 to 2 years ($LRTI_{0-2}$) and 2 to 4 years ($LRTI_{2-4}$); Ear- nose- and throat-surgery between ages 0 to 2 years (ENT_{0-2}) and 2 to 4 years (ENT_{2-4})

Table 4.3 Independent predictors at age 2 years for asthma at 21 years

	OR (95% CI)	Regression coefficient	Contribution to score
Female gender	1.9 (1.2 - 3.2)	0.66	7
Smoking mother	1.6 (1.0 - 2.7)	0.50	5
$LRTI_{0-2}$	1.9 (1.0 - 3.6)	0.65	7
Atopic parent(s)	2.1 (1.3 - 3.4)	0.74	7

Score = -31 + (7 × female gender) + (5 × smoking mother) + (7 × $LRTI_{0-2}$)+ (7 × atopic parent(s))

AUC 0.66 (0.60 – 0.73)

At 2 years of age female gender (OR 1.9; 95% CI 1.2 – 3.2), smoking mother (OR 1.6; 95% CI 1.0 – 2.7), $LRTI_{0-2}$ (OR 1.9; 95% CI 1.0 – 3.6), and atopic parent(s) (OR 2.1; 95% CI 1.3 – 3.4) were independent predictors of developing asthma in young adulthood. (Table 4.3) The AUC of this clinical model was 0.66; 95% CI 0.60-0.73. The fit of the model was good (p= 0.99).
At 4 years of age female gender (OR 2.1; 95% CI 1.3 – 2.5), smoking mother (OR 1.6; 95% CI 1.0 – 2.6), $LRTI_{2-4}$ (OR 2.4; 95% CI 1.4 – 4.0) , and atopic parent(s) (OR 1.9; 95% CI 1.2 – 3.1) were independent predictors of developing asthma in young adulthood. (Table 4.4) The AUC of this clinical model was 0.68; 95% CI 0.62-0.74. The fit of the model was good (p= 0.93).

The probability of developing asthma can be estimated for each child with the formula given in Tables 4.3 and 4.4. For example, a girl (7 points) of 2 years of age, whose parents don't smoke (0 points), whose mother is asthmatic (7 points) and visiting her general practitioner with recurrent lower respiratory tract symptoms (7 points), has a total score of –31 + 7 + 0 + 7 + 7 = -10 points.
Tables 4.5 and 4.6 show the number of subjects in the cohort with and without asthma across different categories of the risk score. For each arbitrary threshold many children were misclassified. For example, at 2 years of age 210 children (30%) would have been unjustly predicted to be non-asthmatic (38 false negative) or asthmatic (172 false positive) if the threshold was < -19 (Table 4.5).

Discussion

Independent predictors of asthma in young adulthood in children aged 2 to 4 years were female gender, smoking mother, lower respiratory tract illness, and parental atopic disease. The performance of a prognostic model including these parameters in terms of the occurrence of asthma in young adulthood was poor. It was not possible to find a cut off point in the risk score that classified the children satisfactorily. In both models too many children were misclassified for each arbitrary threshold to be of any clinical use.

Table 4.4 Independent predictors at age 4 years for asthma at 21 years

	OR (95% CI)	Regression coefficient	Contribution to score
Female gender	2.1 (1.3 - 2.5)	0.76	8
Smoking mother	1.6 (1.0 - 2.6)	0.49	5
$LRTI_{2-4}$	2.4 (1.4 - 4.0)	0.87	9
Atopic parent(s)	1.9 (1.2 - 3.1)	0.65	7

Score = = -32 + (8 × female gender) + (5 × smoking mother) + (9 × $LRTI_{2-4}$) + (7 × atopic parent(s))

AUC 0.68 (0.62 – 0.74)

Table 4.5 Sensitivity, specificity, predictive value positive, predictive value negative for various thresholds of the risk score developed to identify 2 year old children at risk for asthma at 21 years of age

Cut off in risk score	**Number of children with score < than cut off** (Number of children with asthma)	**Number of children with score ≥ than cut off** (Number of children with asthma)	**Sensi-tivity**	**Speci-ficity**	**Positive predict. value**	**Negative predict. value**
< - 26	140 (8)	507 (72)	90	23	14	94
< - 19	433 (38)	214 (42)	53	70	20	91
< - 12	630 (74)	17 (6)	8	98	35	88

Table 4.6 Sensitivity, specificity, predictive value positive, predictive value negative for various cut off points of the risk score developed to identify 4 year old children at risk for asthma at 21 years of age

Cut off in risk score	**Number of children with score < than cut off** (Number of children with asthma)	**Number of children with score ≥ than cut off** (Number of children with asthma)	**Sensi-tivity**	**Speci-ficity**	**Positive predict. value**	**Negative predict. value**
< - 27	135 (5)	536 (78)	94	28	15	96
< - 20	335 (24)	336 (59)	71	53	18	93
< - 12	611 (67)	60 (16)	19	93	27	89

The independent prognostic factors, which were analysed, are in agreement with other longitudinal cohort studies.[5-8] However, current knowledge of development of asthma from childhood through adolescence is mainly based on etiologic studies describing risk factors. In only one recent report of data from the Belmont cohort study prognostic factors were described.[11] Independent prognostic factors in this report were female gender, having atopy, airway hyper-responsiveness, abnormal lung function (low FEV_1/FVC ratio), recent wheeze and hay fever. The different results of this study as compared to ours can probably be explained by differences in study design. Data of the Belmont cohort were collected at the age of 8-10 years, ours at 2-4 years. Hay fever which tends to develop at school-age could therefore not be included in our model. The Belmont cohort study group used clinical prognostic factors such as lung function and skin-prick tests, whereas our aim was to construct a prognostic model on the basis of easily available characteristics from a child's history. In the primary health care setting predictors are needed which are preferably non-invasive, low in costs, easy to obtain and accurate. Lung function and skin-prick tests do not meet these requirements and are therefore not appropriate for common use in primary health care.

To appreciate the results of this study some limitations should be discussed.
First, factors like atopic sensitization,[18] eczema,[6,7] food allergy,[18] and body mass index[19] might be considered as potentially important predictors of asthma. For example, Eysink et al. recently showed that specific IgE assessment improved the predictive accuracy of their model regarding the prediction of asthma at age 6 years based on history data from ages 0 to 4 years.[20] Since the focus of the original study was on UTRI and otitis media, these aspects could not be included in our study. This lack of information on atopy and atopic features might explain some of the poor discriminative value of our prognostic model.
Second, we could not distinguish viral bronchitis from recurrent bronchiolitis, wheezing or asthma symptoms in childhood as at the time of these evaluations it was uncommon to make such a distinction. Parents had been asked whether their child had visited the doctor because of 1) pneumonia, 2) dyspnoea, 3) asthma, or 4) chronic bronchitis. To reduce misclassification as much as possible, we combined these questions into one definition of lower respiratory tract illness (LRTI).
Third, the incidence of asthma in young adulthood was assessed by questionnaires only. We did not include objective measurements like lung function parameters. The ISAAC Questionnaire, however, is a well established and validated tool to assess atopic disease in the general population.[14]
Fourth, in prognostic research, validation of the model is generally recommended.[21] We did not carry out an external validation study, as the performance of our prediction rule was poor. For the same reason we did not use random bootstrapping or penalized maximum likelihood techniques to adjust for overfitting (i.e. over- optimistic estimates of the regression coefficients of the prediction model).[22]

The major strength of our study is the prospective design and the long follow-up period, i.e. from 0 to 21 years of age. The fact that only 693 of the original 1328 study members were evaluated at 21 years of age, may have caused bias, e.g.

study members with asthma may have been most motivated to attend the follow-up visits. Besides it is known that being a student implies a lower response rate.[23] The most important reason for loss to follow-up, however, was that study members could not be traced because they had moved. Moreover, a comparison of the baseline characteristics between the study members and those of the original cohort showed no differences. We therefore believe that selection bias can be considered minimal.

In conclusion,
it was found impossible to predict which young children from the general population would and would not develop asthma at young adult age on the basis of history data only. To our knowledge this is the first attempt to define a practical scoring rule to predict the absolute risk of asthma in young adulthood on the basis of childhood characteristics. Although the performance of our model was poor, we propose that this method is further tested as a tool to predict development of asthma. To inform individual patients on prognosis absolute risk factors should be calculated instead of relative risk factors for asthma.

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Chapter 5

Atopic disease and exhaled nitric oxide in an unselected population of young adults

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Atopic disease and exhaled nitric oxide in an unselected population of young adults

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Abstract

Background
Several studies have reported elevated levels of fractional exhaled nitric oxide (FeNO) in atopic subjects, particularly in asthmatics, suggesting that nitric oxide is a marker of bronchial inflammation. However, the independent influence of different atopic entities (eczema, allergic rhinitis and asthma) on FeNO have never been studied in the general population.

Objective
The influence of questionnaire-based diagnosis of atopic diseases, IgE and lung function measurements on FeNO levels was studied.

Methods
Within the birth cohort, the incidence of asthma, allergic rhinitis, and eczema was determined, and off-line FeNO, spirometry and IgE measurements were performed at the age of 21 years.

Results
FeNO measurements were successfully performed in 361 subjects. Median FeNO-levels were significantly higher in those with versus (vs) without eczema (23.6 vs 18.0 ppb; $p<0.0001$), in those with vs without allergic rhinitis (20.7 vs 17.8 ppb; $p=0.0001$), in those with vs without atopic asthma (23.3 vs 18.1 ppb; $p= 0.02$), but not in those with vs without asthma (20.8 vs 18.3 ppb; $p=0.24$). Eczema, allergic rhinitis, smoking, sex and atopic sensitization appeared to be independently associated with log FeNO in this population sample, while (atopic) asthma was not. No effect on FeNO was observed for lung function parameters.

Conclusion
We conclude that eczema, allergic rhinitis and atopic status, but not (atopic) asthma were independent determinants of FeNO. Future studies into the role of FeNO in asthma should consider the influence of atopic disease outside the lungs.

Introduction

Although concentrations of fractional exhaled nitric oxide (FeNO) are highly variable in healthy subjects, levels outside the normal range are associated with respiratory disease. Patients with asthma show increased levels of FeNO compared with healthy controls[1-4], and so do patients with allergic rhinitis[4,5], COPD[6,7], bronchiectasis[8], pulmonary sarcoidosis[9], active fibrosing alveolitis[10], and acute lung allograft rejection.[11] In contrast, low levels of FeNO have been reported in patients with primary ciliary dyskinesia and cystic fibrosis.[12,13]
Most patients with asthma are sensitized to common aeroallergens. Beside airway hyper responsiveness many have symptoms of eczema or allergic rhinitis, conditions characterised by local activation of macrophages and eosinophils. It has been suggested that FeNO reflects allergic inflammatory activity of the airways, with FeNO levels depending on the degree of atopy.[2]
Although numerous studies have been conducted on patients with atopic asthma and atopic sensitization, the independent effects of different atopic entities (eczema, allergic rhinitis and asthma) on FeNO have not been studied in an unselected population of adults so far. Several studies have descibed determinants of FeNO in community-based samples of children.[2,5,14-16] Determinants of FeNO have been described for young adults as well[17], but eczema was not taken into account in this study.
We therefore measured FeNO in a large birth cohort of young adults and related questionnaire-based diagnosis of atopic disease, IgE and lung function to FeNO values.

Methods

Subjects
This study was part of a follow-up on otitis media of a birth cohort of 1328 children, born in the city of Nijmegen, the Netherlands, between September 1982 and August 1983.[18] In 2004, the home addresses of 1055 of the original cohort members could be traced and 693 subjects completed a questionnaire.[19] These subjects were invited to participate in FeNO, IgE and lung function measurements. All participants gave written informed consent and the study was approved by the medical ethics committee of the University Medical Centre Utrecht.

M*easurement of fractional exhaled Nitric Oxide*
FeNO measurements were performed with standardized offline bag collection with a dynamic flow restrictor.[20,21] Subjects were instructed to inhale through a nitric oxide filter in order to remove ambient nitric oxide (NO). Subsequently, they were asked to exhale through the dynamic flow restrictor, with a target mouth pressure of 15 cmH_2O. This resulted in a flow limitation of 50 mL/s, in accordance with recommendations of the American Thoracic Society (ATS) and European Respiratory Society for FeNO measurement.[22] Contamination of FeNO with nasal NO (nNO) was prevented by exhalation against a positive mouth pressure to ensure closure of the soft palate. After 5 seconds of exhalation of dead space air, a 150 mL Mylar bag was filled. After a resting

period of at least 30 seconds the second bag was filled, according to the same procedure.
Within eight hours the collected air samples were analysed using the NIOX® system (Aerocrine AB, Stockholm, Sweden). FeNO values were expressed as parts per billion (ppb). Two air samples were taken from each bag. Data were reported as mean values from the collected samples. Calibration was performed routinely.

Determinants
Data on eczema, allergic rhinitis and asthma were collected using the Core Questionnaire of the International Study of Asthma and Allergies in Childhood (ISAAC).[23] Eczema was defined as itchy skin rash that has been coming and going for at least 6 months and affecting particular skin sites (i.e. folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes) in the last 12 months. Allergic rhinitis was defined as the presence of a runny or blocked nose in the last 12 months without having a cold or flu, accompanied by itchy-watery eyes and/or some interference with daily activities by this nose problem. Asthma was defined as the occurrence of wheezing in the last 12 months and/or a doctor's diagnosis of asthma in that same period and/or current use of asthma medication. Atopic asthma was defined as asthma plus allergic sensitization, i.e. positive Phadiatop.Parental atopic disease was defined as the occurrence of asthma, allergic rhinitis or eczema in at least one parent.
Data about other possible determinants of FeNO values were also collected, such as height, bodyweight, current use of asthma medication and inhaled corticosteroids (ICS), smoking behaviour in the last year, recent colds and lung function parameters. A recent cold was defined as a cold or flu in the last 4 weeks. Spirometry was performed using the Lilly pneumotachometer system (Viasys healthcare, Masterscreen, Hochberg, Germany). Three technically acceptable maximal flow volume curves were obtained. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were derived from the best curve according to ATS criteria.[23] Subjects were informed not to use a short-acting bronchodilator 6 hours and/or a long-acting bronchodilator 36 hours prior to spirometry.
Serum samples were taken and stored at -20 C until analysis. Total IgE and specific IgE was determined by a solid phase immunometric assay on an ImmunoCAP 250 system of Pharmacia (Pharmacia Diagnostics AB, Sweden).
Specific IgE against a mixture of 10 common aeroallergens, like birch, timothy, cat, dog, horse, and *Dermatophagoides pteronyssinus* was determined by Phadiatop (Pharmacia Diagnostics AB, Sweden), which measures IgE antibodies in serum. Results were expressed as positive (atopic) or negative (non-atopic).

Data analysis
FeNO values were $\log^{10}$ transformed, as they were skewed (Kolmogorov-Smirnov p-value<0.0001), and geometric mean (95% confidence intervals (CI)) and medians (interquartile range) were calculated. Mann-Whitney U tests were used to study the association between FeNO and sex, eczema, allergic rhinitis, asthma, atopic asthma, parental atopic disease, asthma medication, inhalation

corticosteroids, smoking, recent cold, height, body mass index (BMI), FEV_1, FVC, IgE and Phadiatop.
Multiple linear regression models were used to study the independent effect of all variables with a p-value <0.10.

Results

A total of 406 subjects agreed to participate in this study. FeNO could not be measured reliably in 26 subjects due to technical problems. In another 19 subjects measurements were considered invalid and therefore excluded. Reasons were: subjects were unable to exhale at a constant flow rate or there was a significant difference between FeNO values in the two samples according to the ATS/ERS criteria.[22] Participant characteristics are shown in Table 5.1.
According to our definitions, 13% of the participants met the criteria for asthma, 8% for atopic asthma, 34% for allergic rhinitis and 14% for eczema. FeNO values were skewed (Fig 1) but log-normally distributed (Fig 2). The mean log FeNO level was 1.28 (SD 0.20) and the geometric mean FeNO value was 19.1 (95% CI 7.7 – 47.0) ppb.
Median FeNO-levels were significantly higher in subjects with eczema than in those without (23.6 versus 18.0 ppb; p<0.0001), in those with versus without allergic rhinitis (20.7 versus 17.8 ppb; p=0.001), in those with versus without atopic asthma (23.3 versus 18.1 ppb; p=0.02), in men versus women (20.4 versus 17.4 ppb; p<0.0001), in those with elevated versus non-elevated IgE (19.9 versus 16.7 ppb; p=0.001) and in those with positive versus negative Phadiatop (21.6 versus 17.3 ppb; p<0.0001). Lower FeNO-levels were observed in smokers versus non-smokers (16.9 versus 20.0 ppb; p<0.0001) and subjects with a height below 173 cm versus those above 173 cm (18.1 versus 19.3 ppb; p=0.03) (Table 5.2). FeNO was not associated with asthma, BMI, asthma medication, lung function (i.e. FEV_1 and FVC) or recent colds.
In the multivariate linear regression model allergic rhinitis, eczema, sex, smoking cigarettes, IgE and Phadiatop appeared to be independently associated with log FeNO levels (Table 5.3), while atopic asthma, current use of asthma medication and height were not.

Discussion

In this study, we observed an independent effect of atopy, eczema and allergic rhinitis on FeNO levels, but not for (atopic) asthma. Only a few subjects used asthma medication and/or ICS, neither of these determinants were found to alter FeNO values. Sex, current smoking and height appeared to influence FeNO. However, after adjusting height for sex in the multivariate model, no significant relationship was observed, suggesting that higher FeNO levels in men are responsible for the relationship between FeNO and height. Higher FeNO levels in men could be due to a relatively larger lung volume. However, we performed a additional analysis which showed no correlation between the absolute FVC (in litres) and FeNO levels (data not shown).

Table 5.1 Participant characteristics

	All participants ***(n=361)***	**Male** ***(n=137)***	**Female** ***(n=224)***
	N (%)	N (%)	N(%)
Eczema	52 (14)	17 (13)	35 (16)
Allergic rhinitis	122 (34)	44 (32)	78 (35)
Asthma	48 (13)	14 (10)	34 (15)
Atopic asthma	29 (8)	13 (10)	16 (7)
Current astma medication	22 (6)	7 (5)	15 (7)
Current use ICS*	7 (2)	3 (2)	4 (2)
Parental atopic disease	174 (48)	64 (47)	110 (49)
Recent cold	79 (22)	25 (18)	54 (24)
Smoking last 12 months	145 (40)	50 (37)	95 (43)
Phadiatop positive	144 (41)	74 (54)	70 (33)
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Height (cm)	173.6 (9.5)	182.2 (6.5)	168.3 (6.9)
Body mass index (kg/m^2)	23.7 (4.1)	23.7 (3.5)	23.7 (4.4)
FEV_1 (%predicted)	115.8 (14.3)	116.2 (14.1)	115.5 (14.5)
FVC (%predicted)	113.0 (13.2)	115.0 (11.9)	111.8 (13.8)

Table 5.2 Exhaled nitric oxide in different subgroups. Comparisons between median values in the groups were made using Mann-Whitney's U test. Height, BMI, FEV_1, FVC, and IgE were dichotomised using the median value as cut-off point

	Geometric mean FeNO (95% CI) (in ppb)	**Median FeNO** (interquartile range) (in ppb)	p-value
Sex			
Men	20.9 (8.5 – 51.2)	20.4 (14.9 – 29.2)	
Women	17.5 (7.9 – 38.4)	17.4 (13.5 – 23.4)	<0.0001
Eczema			
Yes	23.3 (9.4 – 57.6)	23.6 (16.2 – 34.1)	
No	18.0 (9.4 – 44.5)	18.0 (13.9 – 23.7)	<0.0001
Allergic rhinitis			
Yes	20.6 (8.2 – 51.9)	20.7 (15.1 – 31.3)	
No	17.9 (8.1 – 39.6)	17.8 (13.9 – 23.5)	0.001
Smoking			
Yes	16.8 (7.4 – 38.1)	16.9 (12.4 – 22.5)	
No	20.2 (8.7 – 46.6)	20.0 (14.9 – 26.2)	<0.0001
Heigth			
<173 cm	17.7 (8.1 – 39.0)	18.1 (13.2 – 23.6)	
≥ 173 cm	19.8 (8.1 – 48.4)	19.3 (14.6 – 26.2)	0.03
Asthma			
Yes	20.2 (8.1 – 50.4)	20.8 (13.7 – 27.8)	
No	18.5 (8.0 – 42.8)	18.3 (14.0 – 23.9)	0.24
Atopic asthma			
Yes	22.8 (8.2 – 63.6)	23.3 (15.3 – 31.0)	
No	18.4 (8.0 – 42.1)	18.1 (14.0 – 23.9)	0.02
Atopic Parents			
Yes	19.5 (8.3 – 45.6)	20.0 (14.9 – 26.1)	
No	18.1 (7.6 – 42.2)	17.7 (13.5 – 23.7)	0.02
Asthma medication			
Yes	21.5 (8.2 – 56.4)	21.3 (16.9 – 28.3)	
No	18.6 (8.0 - 43.1)	18.2 (14.0 – 25.1)	0.11
Recent cold			
Yes	18.9 (8.0 – 44.6)	18.6 (14.1 – 24.8)	
No	18.7 (8.0 – 43.7)	18.7 (14.0 – 24.9)	0.72
Body mass index			
<23	19.0 (8.1 – 44.5)	19.0 (14.0 – 24.1)	
≥ 23	18.4 (7.8 – 43.0)	18.3 (14.0 – 25.1)	0.63
FEV_1 %predicted			
<116	19.4 (7.7 – 49.0)	19.0 (14.1 - 27.0)	
≥ 116	18.1 (8.4 – 38.9)	18.5 (13.8 – 23.7)	0.16

Table 5.2 Continued

	Geometric mean FeNO (95% CI) (in ppb)	**Median FeNO** (interquartile range) (in ppb)	p-value
FVC %predicted			
< 112	18.5 (7.8 – 44.0)	18.7 (14.0 – 24.9)	
≥ 112	19.1 (8.4 – 43.8)	19.1 (14.4 – 25.0)	0.62
Serum IgE			
<56	17.4 (8.3 – 36.6)	16.7 (13.9 – 22.6)	
≥ 56	20.3 (8.0 – 51.4)	19.9 (14.7 – 29.0)	0.001
Phadiatop			
Positive	21.5 (8.0 – 54.9)	21.6 (14.7 – 30.9)	
Negative	17.4 (8.3 – 36.2)	17.3 (13.9 – 22.0)	<0.0001

Normal distribution of log mean exhaled nitric oxide values.

Table 5.3 Final multivariate linear regression model with log FeNO as the dependent variable and allergic rhinitis, eczema, smoking, sex, IgE and phadiatop as independent variables.

	B	**SE**	**p-value**
	1.54	0.083	
Allergic rhinitis	-0.053	0.022	0.02
Eczema	-0.067	0.029	0.02
Smoking	0.078	0.020	<0.0001
Sex	-0.076	0.021	<0.0001
IgE	0.00007	0.00001	0.001
Phadiatop	-0.04	0.022	0.06

Eczema appeared to be an independent determinant of FeNO levels in young adults. In literature only data on the relationship between eczema and FeNO in children are available and outcomes are contradicting.[5,15,16,25,26] Franklin et al. found that children with doctors-diagnosed eczema had elevated levels of FeNO.[26] However, Buchvald et al. did not find elevated FeNO levels among children with eczema[5], and Nordvall et al. reported a positive association between FeNO levels and eczema in a general population cohort of children aged 13-14 years, but in a multivariate model this relationship was no longer significant.[16]
Allergic rhinitis was another independent determinant of FeNO levels in this study. Association of allergic rhinitis and FeNO has previously been reported for either atopic, non-asthmatic children and atopic adults.[2,4,5,14,16]
Interestingly, unlike other studies[1-4], we did not find an association between asthma and FeNO. FeNO levels were elevated in atopic asthmatics, but in the regression analysis this determinant appeared not to be independent. Salome et al. already found that FeNO in young adults was associated with asthma, defined as bronchial hyper responsiveness and recent symptoms.[17] Franklin et al. also found that FeNO was associated with atopy, hyper responsiveness and blood eosinophil count in asthmatic children, but not with physician diagnosed asthma.[26] Some therefore suggest that FeNO reflects allergic inflammatory activity of the airways, depending on the degree of atopy[2], whereas others suggest that FeNO is a marker of eosinophilic inflammation, irrespective of atopy.[27]

Most studies into the relationship between asthma and FeNO did not investigate co-existence of atopic entities like eczema and allergic rhinitis. The independent influence of these entities on FeNO levels in our unselected population, regardless of atopy, suggests that the elevated FeNO levels in patients with atopic asthma found by others might be secondary to concomitant eczema or allergic rhinitis.
The overlapping pathofysiology of asthma en allergic rhinitis, the so-called ´one airway, one disease´-concept, supports this hypothesis. Inflammatory mediators as histamine and especially leukotrienes play an important role in inflammation of both the upper and lower airways.[28] However, it is not clear how eczema would fit in this concept. Nitric oxide is synthesised from L-arginine by the enzyme nitric oxide synthase in many cells of the body.[29] It is a free radical gas which is fairly stable, but in tissues it is too short-lived to act at greater distances. An increased level of FeNO may be caused by induction of inducible nitric oxide synthase (iNOS) expression in residential cells, but also by attraction of NO producing inflammatory cells, like mast cells[30], eosinophils[31], macrophages and neutrophils.[32] Increased FeNO levels in patients with allergic rhinitis and eczema might be due to endobronchial release of NO as a manifestation of allergen-induced inflammation in the nose or the skin.

To appreciate the results of our study, several limitations should be mentioned. First, a substantial number of participants in our study were tested in the pollen-season. This may have influenced the results since FeNO levels appear to increase after allergen-challenge in sensitizised individuals.[4]
Second, some subjects had some extreme high FeNO values, which might have influenced our results. A sensitivity analysis (data not shown), in which we excluded outliers with FeNO >55 ppb, did not influence our results.
Third, the subjects who participated in the follow up (N=406) might not be representative for the entire birth cohort (N=1328). Furthermore, we noticed an increased number of participating females, for which we have no explanation.
The major strength of our study is that we had the unique opportunity to study FeNO values in a large group of young adults of the general population. In this study eczema, allergic rhinitis and atopy, but not (atopic) asthma were independent determinants of FeNO. The results shed new light on the value of FeNO in the diagnosis of atopic disease.

Future studies into the role of FeNO in asthma should consider the role of atopic features outside the lungs.

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Chapter 6

Persistence of upper respiratory tract infections in a cohort followed from childhood to adulthood

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Persistence of upper respiratory tract infections in a cohort followed from childhood to adulthood

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Abstract

Objective
To assess (1) prevalences of recurrent URTIs (rURTIs) and relapsing/persistent rURTIs and associated medical consumption between 0 and 21 years of age and (2) whether rURTIs experienced in early life predispose to upper airway disease later in life.

Methods
A cohort of all children born in Nijmegen, The Netherlands, between September 1982 and September 1983, was assessed repeatedly from 2 to 21 years of age with questionnaires regarding infections of the upper respiratory tract (URTIs), use of antibiotics, ENT operations and known risk factors for URTIs.

Results
One hundred and sixty-one of the 693 cohort member (23%) suffered from relapsing rURTIs between 0 and 21 years of age, whereas only 7 (1%) suffered from persistent rURTIs throughout this period. Two hundred and six (30%) had used antibiotics more than once; and 220 (32%) had undergone at least one ENT operation. Of the 166 participants with rURTI between 8 and 21 years, 140 (84%) had had rURTI before.

Conclusions
rURTIs are highly prevalent throughout early life and associated medical consumption is substantial. The challenge therefore is to develop therapeutic/preventive strategies that will prevent rURTIs in the first years of life.

Introduction

Infections of the upper respiratory tract (URTIs), presenting as the common cold, rhinosinusitis, tonsillopharyngitis or otitis media are highly prevalent among young children.[1-3] These infections not only have an impact on children's health and well-being, but also generate high medical costs and indirect costs for the family and the society.[4,5] Children experience, on average, 4–6 URTIs per year.[6] When they grow older, the incidence of these infections decreases, probably as a result of a more mature immune defence and improved anatomical conditions, for instance, of the Eustachian tube. A subgroup of children, however, will develop persistent URTIs.

Population studies have provided important information on the epidemiology of URTIs in children. Little is known, however, about its persistence through adolescence since most of these studies have focused on the period between 0 and 6 years of life and follow-up was restricted to a few years.[1,2,7] For example, it is unknown whether recurrent URTIs (rURTIs) experienced in early life tend to persist through adolescence and whether rURTIs in adulthood are related to recurrent infections earlier in life.

In a large prospective population-based cohort of Dutch children followed from 2–21 years of age, we assessed (1) prevalences of rURTIs and relapsing/persistent rURTIs and associated medical consumption between 0 and 21 years of age and (2) whether rURTIs experienced in early life tend to persist through adolescence.

Methods

Participants

The study members were part of a cohort of 1439 children born in Nijmegen, The Netherlands, between September 1982 and September 1983. Of these children, 1328 participated in the first assessment at 2 years of age, including the history of upper respiratory tract infections and medical consumption (i.e. use of antibiotics due to URTIs and ENT operations) in the first 2 years of life.

Between ages 2 and 4 years, children were visited at home every 3 months by a trained study nurse. At each visit upper respiratory tract infections during the previous 3 months were documented and tympanometry was performed.[8] At the age of 8 years children were re-evaluated and a history of upper respiratory disease experienced between 4 and 8 years was taken.[9] At 21 years of age, the home addresses of the cohort members were traced via the City Council of Nijmegen and a standardized respiratory disease questionnaire (covering the period between 8 and 21 years of age) was sent.

The study was approved by the medical ethical committee of the University Medical Center, Utrecht.

Outcome measurements

Recurrent infections of the upper respiratory tract before the age of 2 years ($rURTI_{0–2}$) were defined as the occurrence of four or more ear infections (otalgia with fever and/or otorrhoea) and/or four or more throat infections over that period and/or six or more common colds in the past 12 months.

Figure 6.1 Flow chart of the assessments of the birth cohort

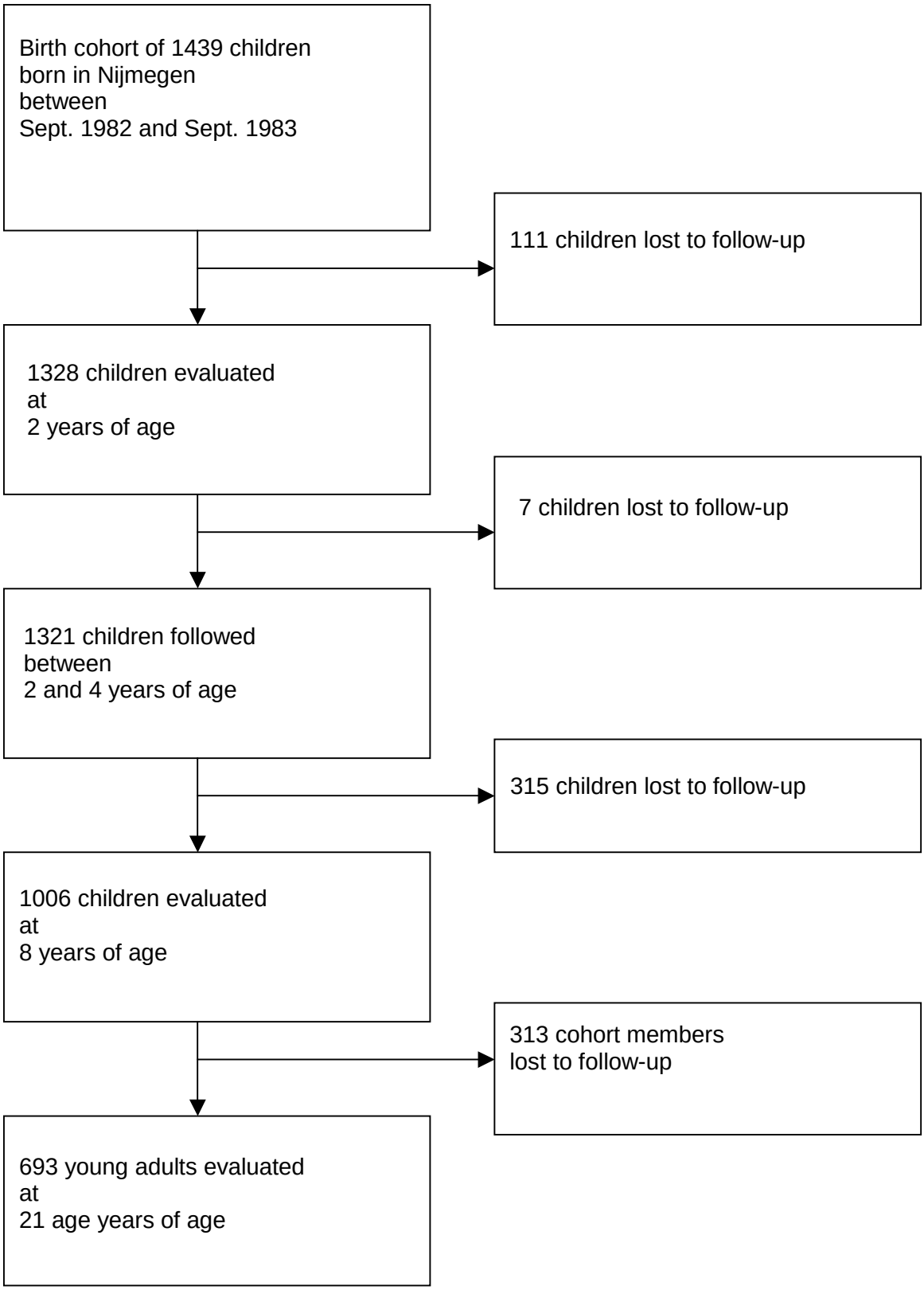
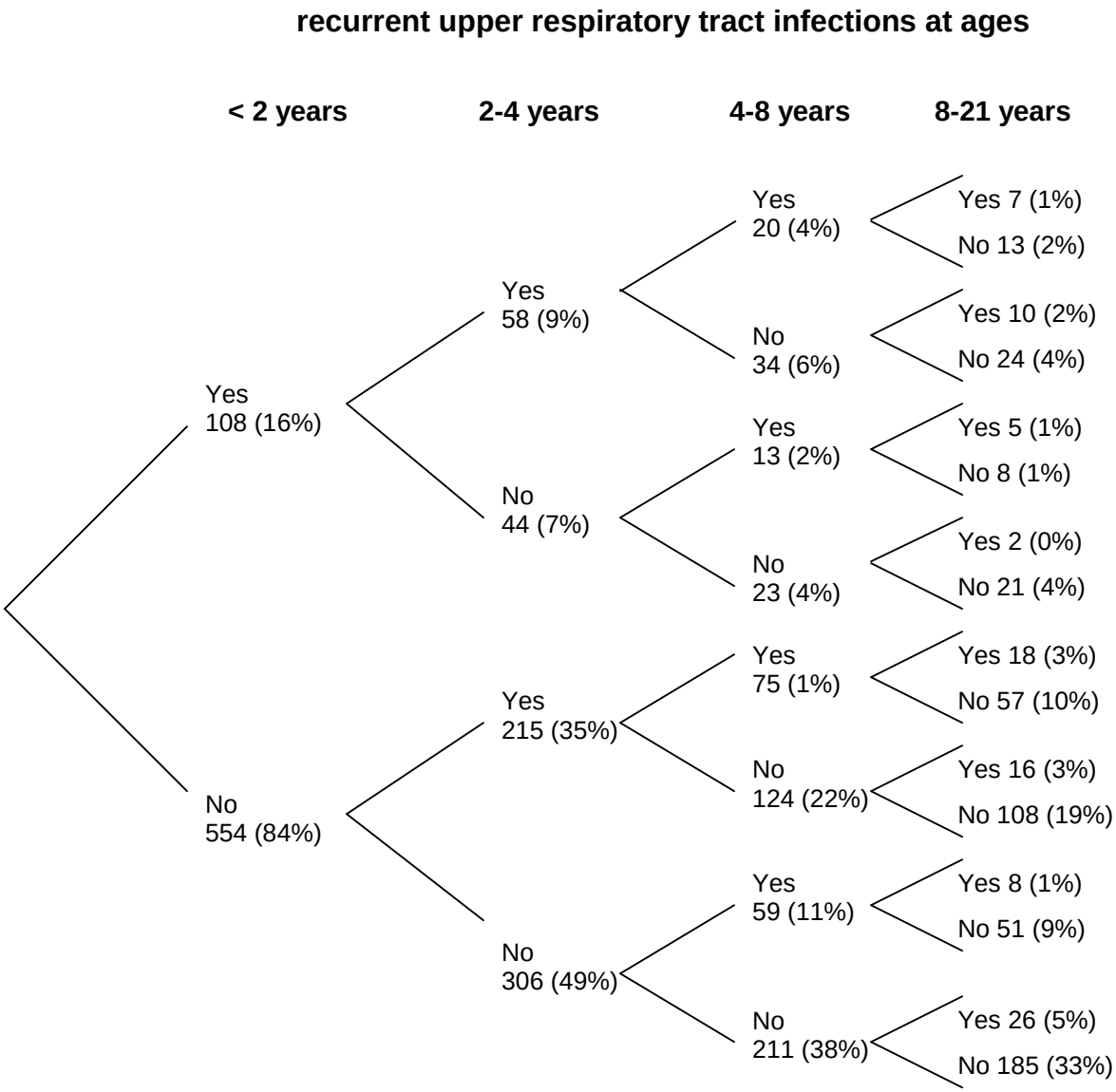


Figure 6.2 Flow chart of the course of recurrent URTIs between 2 and 21 years of age



The numbers do not add to 693 due to missing values with respect tor URTI at each assessment.

At ages 2 - 4 years, the number of 3-monthly episodes in which the child had experienced an upper respiratory tract infection was counted. Recurrent infections of the upper respiratory tract ($rURTI_{2–4}$) were defined as at least four 3-monthly episodes with an ear infection and/or throat infections and/or at least six episodes with a severe common cold. Recurrent infections of the upper respiratory tract between ages 4 and 8 years ($rURTI_{4–8}$) were defined as the occurrence of four or more ear infections and/or four or more throat infections and/or six or more severe common colds.
Recurrent infections of the upper respiratory tract between ages 8 and 21 years ($rURTI_{8–21}$) were defined as the occurrence of four or more ear infections and/or four or more throat infections and/or six or more episodes of common cold or flu like symptoms with fever and/or four or more episodes of sinusitis.
The cut-off points for recurrent infections of the upper respiratory tract were predetermined at the 75th-percentile of the distribution of each infection at each assessment.
Antibiotic treatment for upper respiratory tract infections before the age of 2 years, between ages 2 and 4, 4 and 8, and 8 and 21 years, was defined as at least one course of antibiotics in the corresponding periods. ENT surgery—insertion of tympanostomy tubes, adenoidectomy, tonsillectomy, or any other ENT operation was documented for the same periods.
Relapsing rURTIs was defined as two or more assessments with rURTIs between 0 and 21 years of age, whereas persistent rURTIs was defined as rURTIs at all four assessments.

Statistical analyses
The characteristics of the study members, prevalences of rURTIs between 0 and 2, 2 and 4, 4 and 8, and 8 and 21 years of life, relapsing/persistent rURTIs, antibiotic use and ENT surgery were described with summary statistics. The data were analysed with SAS version 8.0 (SAS Institute, Cary, NC, USA).

Results

Study sample
At the age of 2 years, 1328 children of the birth cohort participated, at 2–4 years from 1321 (99%), at 8 years 1006 children (76%), and at 21 years 693 (52%) study members completed the questionnaires. (Figure 6.1)

Representativeness of the sample
There were no significant differences in sex, history of breast feeding, family size, day care attendance, parental smoking habits, maternal educational level and, most important, the number of recurrent upper respiratory tract infections in the first 2 years of life between the 693 study members followed through 21 years of life and the original cohort of 1328 children (Table 6.1) The most important reason for loss to follow-up was that study members had moved and could not be traced.

Table 6.1 Characteristics of the 693 study participants compared with those of the original birth cohort

Characteristic	Prevalence in study members (N=693)	Prevalence in birth cohort (N=1328)
	% (no. of study members)	
Male sex	46.0 (319)	50.2 (666)
Day care attendance	70.3 (428)	66.8 (744)
Passive smoking (mother)	44.2 (299)	42.1 (539)
Breast fed	69.9 (453)	69.1 (836)
Older sibs	45.3 (294)	46.3 (563)
Recurrent URTI at age 0-2 years	44.9 (297)	44.1 (550)
Antibiotics for URTI at age 0-2 years	21.4 (148)	20.6 (274)

Table 6.2 Outcomes at each assessment among the 693 study members

Outcome	% (no. of study members)
Upper respiratory tract infections	
rURTIs 0 to 2 years[a]	16.3 (108)
rURTIs 2 to 4 years	43.3 (280)
rURTIs 4 to 8 years	29.9 (178)
rURTIs 8 to 21 years	16.5 (114)
Relapsing[b]	23.2 (161)
Persistent	1 (7)
No recurrent URTIs	33.1 (185)
Antibiotics for URTI	
0 to 2 years	21.4 (148)
2 to 4 years	41.6 (269)
4 to 8 years	23.9 (142)
8 to 21 years	36.0 (247)
≥ 2 prescriptions	29.7 (206)
Never	22.4 (155)
ENT-operation	
0 to 2 years	2.7 (19)
2 to 4 years	19.9 (129)
4 to 8 years	17.7 (105)
8 to 21 years	20.2 (139)
≥ 1 ENT-operation	31.7 (220)
≥ 2 ENT-operations	13.7 (95)
Never	50.9 (353)

[a] rURTI = recurrent upper respiratory tract infections, which was defined as the occurrence of at least four ear infections (otalgia with fever and/or otorrhoea) and/or at least four throat infections over that period and/or at least six common cold or flue-like symptoms with fever.
[b] Relapsing URTIs was defined as two or more assessments with recurrent URTIs between 0 and 21 years of age.

Infections of the upper respiratory tract
By the age of 21 years, 67% of the study members had experienced rURTIs during at least one of the study periods; 23% had suffered from relapsing rURTI and 1% from persistent rURTIs (Table 6.2).

Figure 6.2 shows the course of rURTIs between 0 and 21 years of age. One hundred and eighty-five (33%) cohort members reported no rURTIs at all and 26 (5%) had rURTIs between 8 and 21 years without having suffered from such infections at an early age.

Antibiotic prescription
By the age of 21 years, 68% of the study members had been treated with antibiotics for upper respiratory tract infections in at least one age period and 30% had used antibiotics twice or more (Table 6.2).

ENT surgery
By the age of 21 years, 32% of the study members had undergone at least one ENT operation and 14% had undergone two or more ENT operations. Adenoidectomy and the insertion of tympanostomy tubes were the operations most often performed.

Discussion

We found that in an unselected Dutch birth cohort followed up to 21 years of age, 23% of the participants suffered from relapsing/persistent rURTIs through these ages. About 30% received antibiotics for this indication more than once and the same proportion underwent an ENT operation. Incidence of rURTIs was highest (43%) between ages 2 and 4 years. Of the 166 participants with rURTI between 8 and 21 years, 140 (84%) had had rURTI before.

Although high prevalences of URTIs during early childhood have been reported before,[1-7] no one has followed its natural course into adulthood. Such information is essential to understand the consequences of these infections in early life regarding upper airway disease and associated medical consumption later in life. This information might also give clues for developing more effective therapeutic and preventive strategies. One in three members of the birth cohort had received antibiotics for upper respiratory tract infections repeatedly. This indicates that, even in The Netherlands, where a restrictive use of antibiotics has been practiced for several decades,[10,11] URTIs account for a sizable proportion of total antibiotic prescription. Similarly, one in three study members had undergone ENT surgery, reflecting high expectations of doctors and patients regarding surgical management of URTIs in The Netherlands.[11,12] Surgical rates may be slightly larger than in the population at large, since all cohort members had undergone regular ENT examinations, specifically for otitis media with effusion, a condition that often remains unnoticed unless actively screened for.

The major strength of our study is the prospective design and the long follow-up period, i.e. from 0 to 21 years of age. Only 693 of the original 1328 study members, however, were evaluated up to 21 years of age and could be included in the final analysis. This could have caused a bias if the study members who were lost to follow-up formed a selective group, e.g. if study members with rURTIs had been most motivated to attend the follow-up visits. This might have resulted in an overestimation of the prevalence of relapsing/persistent rURTIs and associated medical consumption. The most important reason for loss to follow-up, however, was that a study member could not be traced because he or she had moved. Moreover, a comparison of the baseline characteristics of the study members with complete data and those of the original cohort showed no difference between both groups. We therefore believe that selection bias was minimal.

Some further limitations deserve to be mentioned.
First, we have not taken into account seasonal factors in our analyses, whereas it is known that the time of year a questionnaire is administered is important with respect to URTIs.[13,14] However, as our study is a birth cohort study in which the participants were seen at scheduled follow-up visits, all seasons are represented so that information bias due to a seasonal effect will be minimal.
Second, information on infections of the upper respiratory tract and related environmental factors were based on parental and self-report at ages 2, between 2 and 4, and at 8 and 21 years. It is possible that this information was not accurate enough and therefore some misclassification regarding URTIs cannot be excluded. For example, parents of children with frequent infections at an early stage may be more vigilant for these infections later in life.
Third, experimental studies have shown that many upper respiratory tract infections in children go unnoticed. Some authors therefore claim that these kind of epidemiological studies underestimate the true frequency of affected individuals in the population.[15] However, if parents do not recall certain infections of the upper respiratory tract, they are most likely to be mild and therefore less relevant. More importantly, we have avoided the problem of consultation bias, i.e. some parents never visit a doctor for an URTI, whereas others bring their child to the GP for minor reasons. We therefore believe that the data of our study do provide the useful clinical information to doctors involved in the care of children with upper respiratory disease.

In summary,
rURTIs are highly prevalent, especially between 2 and 4 years and associated medical consumption is substantial. The challenge therefore is to develop therapeutic and preventive strategies that will prevent rURTIs in the first years of life.

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Chapter 7

Dissociation between questionnaire, lung function, atopy and airway inflammation parameters in a general population of young adults

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Dissociation between questionnaire, lung function, atopy and airway inflammation parameters in a general population of young adults

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Abstract

Background
Data on the association between reported items and objective measures of asthma and atopy are scarce. Using factor analysis to formulate scores of increased reliability and validity, we studied how the dimension underlying self-reported subjective symptoms of asthma and atopic disease might relate to measures of lung function, IgE and allergic airway inflammation in the general population.

Methods
Of an original birth cohort 406 subjects completed a written questionnaire, performed lung function tests and measurements of exhaled bronchial nitric oxide (FeNO), serum IgE, and Phadiatop at the age of 21 years. Factor analysis was used to relate various features of asthma and atopic disease into separate, complementary domains without a priori assumptions. Multivariate regression analyses were performed to study the associations between subjective self-reported symptoms and objective measures.

Results
Factor analysis revealed two objective factors consisting of 1) pulmonary function and 2) serum IgE, Phadiatop and FeNO measures and explaining 62% of total variance. Four subjective factors could be defined as 1) asthma symptoms, 2) doctor's diagnosed asthma, 3) allergic rhinitis, 4) parental history of allergic disease, and these explained 55% of the total variance. Objective factor 2 (IgE, Phadiatop and FeNO) was associated with subjective factor 3 (allergic rhinitis) (r=-0.44, 95% CI -0.53; 0.33). The relations between the other subjective and objective factors appeared to be poor.

Conclusions
In this general population cohort, self-reported symptoms were largely independent and separate from objective measures like lung function, IgE, Phadiatop, FeNO.

Introduction

Asthma is recognised as a very heterogeneous syndrome.[1] It comprises recurrent and reversible airflow obstruction, airway hyper responsiveness, allergy and airway inflammation, and these can be measured in many different ways. Clinically, the diagnosis of asthma is based on presented symptoms of wheeze, cough and dyspnoea, and a positive family history. To date, no generally accepted definition of asthma or validated quantitative criteria for clinical and epidemiological purposes have been available. Most epidemiological studies of asthma and allergy rely on reports from subjects or parents of "wheezing during the last 12 months", "doctor's-diagnosed asthma", and/or "the use of bronchodilators or inhaled corticosteroids".[2] Questions used are often drawn from recognised questionnaires, e.g. the International Study of Asthma and Allergy in Children (ISAAC) and the European Community Respiratory Health Survey questionnaires.[3,4] However, the validity and reliability of these question items is not known and there are no generally accepted scoring systems either. Some studies combine questionnaire items with objective measures of lung function, bronchial hyper responsiveness, IgE, and markers of airway inflammation. The implications of such pooling are unclear because the association between the subjective participant-reported items and objective measures has, however, not been studied properly.[5-7] Factor analysis allows reduction of many items of information to a few underlying domains, called 'factors', which are more or less independent. Each factor score is defined by a formula, in effect the sum of the items, weighted to emphasise those that most strongly associate with each other and so with the factor ("high loading"); it is from these that the factor is defined. This technique considerably enhances the reliability of measures relative to that available from single items and so avoids high penalties of adjustment for multiple tests when items are many.

Factor-analytic studies of asthma-related variables have been performed in patients[8-14], but so far not in a general population study. In a longitudinal cohort of young adults, both questionnaire items[15] and pulmonary function measurements, including reversibility testing, measurement of FeNO and markers of atopy (IgE and Phadiatop) at the age of 21 years were available. This cohort offered a unique opportunity to relate these various features into separate, complementary domains without a priori assumptions. This paper addresses whether domains, composed of questionnaire-based items are associated with domains of objective measures like pulmonary function, serum IgE and FeNO measurement.

Methods

Participants were recruited from a prospective birth cohort study on respiratory tract infections of 1328 subjects, born in Nijmegen, the Netherlands, between September 1982 and September 1983.[16,17] At the age of 21 years, cohort members were re-invited for assessment of the presence of asthma and atopic disease.[15] Home addresses were traced via the residence register of the City Council. The ISAAC Core Questionnaire[3], was sent to all cohort members.

Table 7.1 Questionnaire items and objective measures

Questions from questionnaire		Short hand item
Have you ever had asthma?	yes/no	ever asthma
Have you had wheezing or whistling in the chest in the last 12 months?	yes/no	wheeze last 12 mo
Has your chest sounded wheezy during or after exercise in the last 12 months?	yes/no	exercise induced wheeze last 12 mo
Have you had night time dyspnoea in the last 12 months?	yes/no	nighttimes dyspnoea last 12 mo
Have you ever had hay fever?	yes/no	ever hay fever
Have you ever had eczema?	yes/no	ever eczema
Do you have allergy confirmed by an allergy test?	yes/no	confirmed allergy
Has a doctor diagnosed asthma in the last 12 months?	yes/no	doctor's diagnosed asthma last 12 mo
Has a doctor diagnosed asthma ever?	yes/no	doctor's diagnosed asthma ever
Have you had a problem with sneezing, or a runny, or blocked nose, accompanied by itchy-watery eyes, when you did not have a cold or the flu in the last 12 months?	yes/no	hay fever last 12 mo
Have you had an itchy rash which was coming and going for at least six months in the last 12 months?	yes/no	eczema last 12 mo
Does or did your father or mother have asthma?	yes/no	parental asthma
Does or did your father or mother have hay fever or allergic rhinitis?	yes/no	parental hay fever
Does or did your father or mother have eczema?	yes/no	parental eczema
Do you use asthma medication?	yes/no	use asthma medication
Has your chest sounded wheezy during sleep in the last 12 months?	yes/no	wheeze during sleep last 12 mo
Objective measures		
FEV_1		(% predicted)
FEV_1/FVC		(%)
MEF_{50}		(% predicted)
Reversibility, change of %pred FEV_1 after bronchodilation		(%)
FeNO		ppb
Serum IgE		kU/l
Serum Phadiatop		positive/negative

In addition, data on current use of asthma medication, smoking behaviour in the last year and parental history of atopic diseases, i.e. asthma, allergic rhinitis and/or eczema in one of the parents, were obtained.
In 406 participants additional measurements of pulmonary function (pre- and post-bronchodilator FEV_1, FEV_1%FVC), FeNO, IgE and Phadiatop were performed. The medical ethics committee of the University Medical Centre Utrecht approved the study, and all participants gave written informed consent.

Lung function
Spirometry was performed in a mobile lung function lab using the Lilly pneumotachometer system (Jaeger, Masterscreen, Würzburg, Germany) as described before.[18] Subjects were informed not to use short-acting bronchodilator medication for 6 hours and long-acting bronchodilator medication not for 36 hours in advance. Results were compared to gender- and height-related reference values according to the official statement of the European Steel and Coal Community.[19] Spirometry was repeated after inhalation of a bronchodilator (800 µgram salbutamol / Ventolin Diskus®). Reversibility of bronchial obstruction was expressed as the change of FEV1 %predicted.[20]

Nitric Oxide
Fractional exhaled bronchial NO (FeNO) measurements were performed with standardized offline bag collection.[21] Subjects were asked to inhale through a nitric oxide filter in order to remove ambient NO. Subsequently they were asked to exhale with a constant target mouth pressure of 15 mbar. After five seconds of exhalation of dead space air, the (150 Ml) Mylar bag was filled during another 5 seconds. After a resting period of at least 30 seconds the second bag was filled, following the same procedure. Within eight hours the collected air samples were analysed using the NIOX® system (Aerocrine AB, Stockholm, Sweden). Two air samples were taken from each bag. A mean value of four samples (expressed as parts per billion (ppb)) was calculated.
FeNO levels were determined according to the standardized procedure of the American Thoracic Society.[21]

IgE and Phadiatop
Blood sampling was performed via venous puncture. In the serum samples total IgE (kU/l) was determined by a direct paper radioimmunosorbent test (Pharmacia Diagnostics, Sweden). Furthermore the concentration of serum IgE antibodies against a mixture of 10 airborne allergens present in a solid phase was assayed using the Phadiatop test (Pharmacia Diagnostics, Sweden). The results were expressed as positive (atopic) or negative (non-atopic).

Statistical analyses
Statistical analysis included assessment of normality, summarizing descriptives of baseline data and factor analysis. Table 7.1 shows the subjective questionnaire-based items and the objective measures included in the factor analysis.
The number of factors to be extracted was determined by the conventional criterion of the number of principal components exceeding an eigenvalue >1.00. This is an index of the proportion of variance explained by k successive factors

and the criterion serves to ensure that all the factors are genuinely summarising more variance than 1/k % i.e. the expected average for any raw variable. Varimax rotation procedure[22], which was used, increases the interpretability of the factors by rotation to a simple structure with optimally contrasting loadings.
We performed factor analyses separately for report items and for objective measures. Finally, multivariate regression analyses were performed to study the association between the subjective and objective factors.

Table 7.2 Baseline demographic and clinical characteristics of the 406 participants

	All participants (N=406)
	N (%)
Males	155 (38)
Smoking last 12 months	160 (40)
Parent(s) with asthma	62 (15)
Parent(s) with hay fever/allergic rhinitis	124 (31)
Phadiatop positive	169 (42)
	Mean (SD)
Age (yrs)	21.5 (0.6)
FEV1 † (% predicted)	115 (14)
FEV1/FVC‡ (%)	87 (17)
FeNO (ppb) (median (quartile range))	19 (14 – 26)
IgE (kU/l) (median (quartile range))	56 (24 -189)

†FEV_1 = forced expiratory volume in 1 second, expressed as percentage of the predicted reference value; ‡FEV_1/FVC ratio = FEV_1 in litres divided by the forced vital capacity in litres.

Results

Of 1328 original cohort members 693 subjects completed the written questionnaire. Of these, 406 subjects provided complete data on lung function, FeNO and IgE measurements and were included in the present study. Baseline characteristics are presented in table 7.2.

Factor Analysis

Reported items
Barlett's test of sphericity indicated a correlation matrix with sufficient structure for factoring between the used items (chi-sq = 1835.367, degrees of freedom =120, $p < 0.001$). The Kaiser–Meyer–Olkin measure of sampling adequacy was 0.79, showing suitability of the data for factoring. Principal component analysis yielded 4 factors, explaining 55% of variance when the eigenvalue 1.0 criterion

for the last was applied. Factor loading coefficients for each Varimax-rotated factor are displayed in Table 7.3. Asthma symptoms loaded in factor 1, doctor's diagnosed asthma and use of medication in factor 2, hay fever and proven allergy in factor 3 and parental history of atopic disease and eczema in factor 4. Using a cut-off of 0.450 for the loading coefficients no overlap at all was found, and more generally there was relatively little marginal cross-loading between the factors at loadings between 0.250 and 0.450.

Objective measures

Barlett's test of sphericity again indicated sufficient structure for factoring between the used measures (chi-sq = 847.095, degrees of freedom =21, $p < 0.001$). The Kaiser–Meyer–Olkin measure of sampling adequacy was 0.63. Factor analysis yielded 2 separate factors, explaining 62% of the total variance. Factor loading coefficients for each Varimax-rotated factor are displayed in Table 7.4. Lung function loaded in factor 1, Phadiatop, IgE and FeNO in factor 2. Neither overlapping factor loadings nor marginal cross-loadings were found.

Regression analysis

The multivariate regression analyses (Table 7.5) quantify the possible reflection of underlying objective variations within the subjective factors. Only one association appeared to be substantial: objective factor 2 (IgE, Phadiatop and FeNO) with subjective factor 3 (allergic rhinitis) (r=-0.44, 95% CI -0.53; 0.33). Three other of the eight between-factor associations are marginal, but the other four can be considered truly null.

Table 7.3 Varimax rotated factor loading matrix from factor analysis for subjective items

	1	2	3	4
ever asthma	**.712**	.151	**.**214	.125
wheeze during last 12 mo*	**.849**	.168	.159	.016
exercise induced wheeze last 12 mo	**.640**	.340	.184	.065
nighttimes dyspnoea last 12 mo	**.613**	.183	.025	.156
ever hay fever	.132	.083	**.838**	.065
ever eczema	.025	.045	.367	.354
confirmed allergy	.043	.302	**.621**	.198
doctor's diagnosed asthma last 12 mo	.162	**.830**	.047	.017
doctor's diagnosed asthma ever	.254	**.727**	.226	.082
watery or itchy eyes last 12 mo	.256	.031	**.772**	.022
itchy exanthema last 12 mo	.116	-.095	.256	**.482**
parental history asthma	.052	.138	-.068	**.677**
parental history hay fever	.044	.031	.038	**.575**
parental history eczema	.064	.039	.119	**.657**
current asthma medication	.364	**.774**	.081	.062
wheeze during sleep last 12 mo	**.762**	.085	.024	.018

For clarity of reading all items loading >0.45 are in bold; *mo = months

Table 7.4 Varimax rotated factor loading matrix from factor analysis for objective measures

	1	2
FeNO (ppb)*	-.098	**.766**
FEV_1† (% pred)	**.763**	-.003
FEV_1/FVC‡ (%)	**.825**	-.109
reversibility of FEV_1# (%)	**-.658**	.124
MEF_{50}§ (% pred)	**.920**	-.024
serum IgE (kU/l	-.099	**.806**
Phadiatop (positive)	.003	**-.705**

Measures loading >0.45 are in bold;

*FeNO = fractional exhaled nitric oxide expressed in parts per billion (ppb);
†FEV_1 %pred is forced expiratory volume in 1 second, expressed as percentage of the predicted reference value; ‡FEV_1/FVC ratio = FEV_1 in litres divided by the forced vital capacity in litres; #Reversibility = the % change in FEV_1(%pred) (ΔFEV_1 %), after bronchodilation with 800 microgram salbutamol; §MEF_{50} = the forced expiratory volume at 50% of total vital capacity.

Table 7.5 Regression coefficients and their corresponding 95% confidence intervals (95% CI) of the association between the subjective and objective factors

	Subjective 1 Beta (95 % CI)	**Subjective 2** Beta (95 % CI)	**Subjective 3** Beta (95 % CI)	**Subjective 4** Beta (95 % CI)
Objective 1	0.23 (0.13; 0.34)	0.13 (0.03; 0.24)	0.06 (-0.04; 0.16)	-0.02 (-0.13; 0.09)
Objective 2	-0.11 (-0.21; -.003)	-0.07 (-0.17; 0.04)	**-0.44 (-0.54; -0.34)**	-0.08 (-0.19; 0.03)

Multiple regression was used with each subjective variable as one dependent variable and the two objective variables fitted in combination as potential determinants.

Objective factor 1 = 'pulmonary function', objective factor 2 = 'IgE, Phadiatop and FeNO', subjective factor 1 = 'asthma symptoms', subjective factor 2 = 'doctor's diagnosed asthma', subjective factor 3 = 'allergic rhinitis' and subjective factor 4 = 'parental atopic disease'.

Discussion

This is the first study performing factor analyses on various asthma and atopy related variables in the normal range of the population. Four independent domains were defined from reported items and two among the objective measurements. Factor analysis proved to be useful to reduce the large set of variables to a few, relatively independent and reliable factor scores and these were interpretable.
Only objective factor 2 (IgE, Phadiatop and FeNO) and subjective factor 3 (allergic rhinitis and confirmed allergy) appeared to be associated with each other.

Subjective factor structure and loadings
The factor 'asthma symptoms', appeared to be independent of the factor 'doctor's diagnosed asthma'. This suggests severe limitations when attempting to compare studies using asthma symptoms as outcome parameter with studies using doctor diagnosed asthma. Such dissociations in our data depend in part on a statistical technique that encourages independence (factor rotation). However, the fact that it produces plausible sets of items loading on each factor indicates the multiple dimensions of response. Although few clinical studies have used such reported outcomes, it is consistent with one, describing that 'asthma symptoms', and 'severity of asthma' were largely independent of the component 'asthma-specific quality of life'.[12] It remains to be seen which of the factors described here in a normal population replicate in clinical samples, but generally the dimensions found to distinguish mild and severely affected patients also distinguish non-sufferers from patients.

Objective factor structure and loadings
Similar to a study on COPD[23], the present study showed that individual parameters of airflow limitation, traditionally considered to be a mainstay of asthma diagnosis and severity, were combined in one factor, whereas parameters of inflammation, like FeNO, loaded in another factor. This was also shown in a cohort of asthmatics, demonstrating that lung function, bronchial responsiveness with reversibility, and eosinophilic inflammation in sputum were independent dimensions.[11]
FeNO was measured as an easily obtainable marker of airway inflammation that is potentially useful for disease monitoring.[24] Like Leung et al[8] we showed that Phadiatop, IgE and FeNO are loading in the same factor, suggesting that these markers are linked at the pathophysiological level. It has been suggested that FeNO reflects allergic inflammatory activity of the airways, depending on the degree of atopy.[25,26] Thus our objective results also show a dimensionality to that seen in patients with asthma.

Relationship between subjective and objective domains
The very restricted linkage between domains has to be considered a genuine finding of largely weak or null associations in the light of three considerations which make the test a sufficiently sensitive one: (a) similarities of the correlation structures of each domain with those seen in other studies within patient populations; (b) the medium-to-large sample size, and (c) the reliability of

measurement achieved by the data reduction. We are thus forced to conclude that, with exception of the link between inflammation and allergic rhinitis, there is a somewhat wide gulf between the dimensions of asthma and atopy as reported by individuals and several of the currently available biological markers. It is beyond the present scope to debate whether the objective measures are deficient, or patient reports in this area are too arbitrary to be useful clinically or in research; each of these propositions could be partly true.

To set our findings in context some possible limitations should be discussed.
First, we did not measure other markers of asthma, namely bronchial hyper responsiveness (BHR), and sputum or bronchoalveolar lavage, both potentially important components of the asthma and atopy syndrome. It would be interesting to assess a potential relation of BHR to FeNO or eosinophilic inflammation.
Second, the results can not be generalized to patients with asthma since we studied a birth cohort of the general population. On the other hand, our study contributes to the main concept that asthma and atopic disease comprise different independent dimensions of only partially overlapping features, with their own pathophysiological mechanism.

In conclusion our data show that questionnaire-based scores and objective measures of asthma and atopy are separate and largely independent. This confirms the complex heterogeneity of the disease. For diagnosis in epidemiological studies researchers should realize that the association between subjective and objective variables is poor. Factor analysis should be used more widely as a tool to overcome the limited validity and variability of responses to single questionnaire items in epidemiological studies.

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Chapter 8

Summary and Discussion

Walter A.F. Balemans

Summary and Discussion

Summary

The increasing rates of asthma and atopic disease in the last decades[1-3] is unlikely to be explained by genetically determined factors but rather by changing environment and lifestyle factors, like allergen exposure and childhood microbial exposure socioeconomic status and dietary habits.[4] In this thesis we explored the role of childhood respiratory tract infections (RTI) on the development of asthma and atopy. As reviewed in **Chapter 2,** the evidence in favour of the concept that childhood infections protect against atopic development, the so-called 'hygiene hypothesis'[4], is merely based on studies using indirect markers of overall microbial exposure, i.e. crowding of children like attending day care or family size.[5-7] There is, however, no strong evidence that early and symptomatic childhood RTI themselves protect from atopic development. On the contrary, many studies that measured symptomatic respiratory infections in childhood found a positive association between early infections and asthma.[8-13] This may be due to reverse causation, meaning that individuals with a genetic predisposition to develop atopy and asthma are at increased risk to develop symptomatic respiratory symptoms due to viral infections early in life. There is a general lack of longitudinal studies into the association between childhood RTI and the development of atopic disease later in life. In **Chapter 3** we therefore studied a birth cohort that was prospectively followed from 2 to 21 years of age of which data had been collected on childhood RTI.[14,15]

In **Chapter 3A** we describe that recurrent upper respiratory tract infections (URTI) did not decrease the risk of development of atopic disease later in life. The associations between URTI 2 to 4 years and asthma, allergic rhinitis and eczema at 21 years were RR 1.45 (95% CI 0.95 – 2.21), RR 0.99 (95% CI 0.79-1.25), and RR 1.19 (95% CI 0.81 – 1.75), respectively. Similarly, lower respiratory tract illness (LRTI) at early age tended to have an increased risk of asthma in adulthood. The association between LRTI at 2 to 4 years and asthma at 21 years was RR 1.91 (95% CI 1.26 – 2.87). This relation can be causal in either direction, i.e. children experiencing more RTI may be prone to develop subsequent atopic disease, or predisposed atopic children may experience more symptomatic RTI. The latter is supported by our finding of a slightly increased incidence of URTI and LRTI among children of atopic parents (data not shown) and has been described earlier.[16]
Our study is one of the few studies on this subject that followed a cohort from childhood to young adulthood.[17] It should, however, be noted that young adults may have a different asthma phenotype as compared to children.[18,19] Since we do not have data on asthma and atopy in childhood, we could not study the course of asthma and atopy in our participants. Furthermore, the concept of the hygiene hypothesis is not solely explained by differences in the occurrence of recurrent URTI. Exposure to other infections, like gastro-intestinal infections, and exposure to gram-negative micro-organisms, may influence the immune system and lead to altered regulation of the immune response.[20,21] Important

changes in exposure to many of these agents have occurred in the last decades of the former century, particularly in western society, all potentially directing the immune response towards the Th2 pathway and development of atopic disease. Furthermore, some authors have suggested that only infections in the first year influence atopic development, whereas others showed that also exposures after infancy may be of influence.[22] Although in the current study we only have retrospective data on the occurrence of URTI in the first 2 years of life, we could not confirm any association with atopy later in life and URTI either before or after infancy.

Asthma and atopy comprise a heterogeneous complex of features, like symptoms of wheezing and dyspnoea, reversible bronchial obstruction, airway inflammation, elevated serum IgE and sensitization to specific allergens.[23] In many studies the outcome measures are just based on questionnaires,[24-27] leading to definitions that only partly describe the complexity of the disease. For this reason we studied the associations between recurrent childhood RTI and objective measures of atopy and asthma by measurement of pulmonary function, serum IgE, specific IgE to one or more allergens as measured by Phadiatop and exhaled nitric oxide (FeNO). (**Chapter 3B**) In 406 of the 693 participating cohort members allergic sensitization (serum IgE and specific IgE), lung function (FEV_1, FVC and reversibility of bronchial obstruction after bronchodilation) and a marker of allergic airway inflammation (FeNO) was measured at the age of 21 years. Recurrent URTI in childhood appeared not to be associated with markers of allergic sensitization: 45% of the children with URTI between 2 and 4 years had a positive Phadiatiop at 21 years of age versus 41% in those without URTI (p=0.45). Neither were recurrent URTI associated with lung function parameters; the mean FEV_1 predicted was 116.5% in those with URTI between 2 and 4 years versus 115.4% in those without URTI (p=0.99). Remarkably, FeNO measurement, as parameter of allergic inflammation, was slightly decreased in those participants experiencing recurrent URTI before the age of 2 years (17.0 ppb versus 19.5 ppb; p = 0.03) suggesting a protective effect on allergic inflammation. This result could not be confirmed with any associations with other markers of allergy, such as IgE.

Childhood LRTI were not associated with FEV_1, reversibility of bronchial obstruction, elevated IgE, specific IgE and increased FeNO values at age 21 years.

The results seem to confirm that childhood RTI do not increase the risk for development of atopic disease.

Adenoidectomy and tonsillectomy are common surgical procedures in young children with recurrent upper RTI.[28] In **Chapter 3C** we therefore studied the associations between ENT-surgery as proxy marker for recurrent RTI in childhood and atopic disease in young adults. Since adenoidectomy and/or tonsillectomy could lead to reduced humoral and cellular responses, i.e. altering the stimulation of the immune system in young children[29], they may subsequently be associated with atopic disease later in life. Our results showed no associations between ENT-surgery and any of the outcomes measured later in life, i.e. asthma, allergic rhinitis and eczema. (RR 0.93, 95% CI 0.52 – 1.64; RR 0.94, 95% CI 0.68 – 1.30; and RR 1.00, 95% CI 0.59 – 1.68, respectively.) The idea that adenoidectomy and/or tonsillectomy in childhood may be harmful in terms of atopic development could therefore not be confirmed.

A prognostic study was performed to identify independent predictors in early childhood for asthma and atopy later in life (**Chapter 4**). This might be relevant, since early identification of high risk groups might result in better prevention and treatment strategies. In children, aged 2 to 4 years, independent predictors of asthma in young adulthood were female gender, smoking mother, lower respiratory tract illness, and parental atopic disease. However, the performance of the prognostic model including these parameters in terms of the occurrence of asthma in young adulthood was poor. New, well-designed prospective cohort studies are required for better prediction models for children into the development of asthma in adulthood.

In **Chapter 5** we further explored the association between FeNO measurements and questionnaire-based diagnosis of atopic diseases, IgE and lung function measurements. FeNO, a free radical gas produced by residential and inflammatory cells, is a promising parameter of allergic airway inflammation, and easy to measure by means of a non-invasive procedure.[30] Recently, it was found to be valuable in titrating inhaled steroids in children with asthma.[31] Previous studies already described determinants of FeNO in community-based samples of children.[30,32-35] and adults,[36] but in general these studies did not take all atopic entities, like allergic rhinitis and eczema, into account. In our study elevated IgE and positive specific IgE tests, having eczema and allergic rhinitis were independent determinants of elevated FeNO levels, but surprisingly not (atopic) asthma (based on the ISAAC plus positive specific IgE). Although FeNO levels were elevated in atopic asthmatics, the effect disappeared after adjustment for other factors.
The independent influence of allergic rhinitis and eczema on FeNO levels in our unselected population suggests that the elevated FeNO levels in patients with atopic asthma, as found by others,[36] might be secondary to concomitant eczema or allergic rhinitis. This may point to a shared immunological pathway for various atopic features.[37]

In **Chapter 6** we studied the persistence of recurrent URTI (rURTI) from childhood through adulthood and associated medical consumption. Furthermore we assessed whether rURTI experienced in early life predispose to upper airway disease later in life. We found that 23% of our study members suffered from relapsing rURTI between 0 and 21 years of age, whereas only 1% suffered from persistent rURTI. Thirty percent of the study members had used antibiotics more than once between 0 and 21 years; and 32% had undergone at least one ENT-operation. Of the 166 participants with rURTI between 8 and 21 years, 84% had had rURTI before.
These results show that rURTI are highly prevalent throughout early life and associated medical consumption is substantial. The challenge therefore is to develop therapeutic/preventive strategies that will prevent rURTI in the first years of life, e.g. vaccination strategies.

In **Chapter 7** we investigated whether domains, composed of questionnaire-based items were associated with domains of objective measures like pulmonary function (FEV_1, FVC and reversibility of bronchial obstruction), serum IgE (total IgE), specific IgE and FeNO measurement. Using factor

analysis both questionnaire-based items and objective measures of asthma and atopy could be associated into more or less independent factors. Subjective factors comprised: ‘asthma symptoms’, ‘doctor’s diagnosed asthma’, ‘allergic rhinitis’ and ‘parental history of atopic disease’. Objective factors were: ‘lung function’ and ‘IgE, specific IgE and FeNO’. The study showed that questionnaire-based items and objective measures of asthma and atopy appeared to be separate and largely independent dimensions. This underlines the heterogeneity of the disease and importantly, shows the limited relations between the different entities of asthma and atopy. Our study contributes to the main concept that asthma and atopic disease comprise different independent dimensions of only partially overlapping features, with their own pathophysiological mechanism.

Discussion

General discussion and recommendations

The studies presented in this thesis greatly challenge current concepts about “asthma” as a disease entity and have important consequences for future studies into etiology and pathogenesis of this disease.

Our studies clearly demonstrate that asthma is not a simple single disease. Although many studies have simplified asthma to “symptoms of wheeze during the last 12 months”, this thesis has shown that subjective symptoms of asthma often are not related to objective parameters of airway obstruction, airway inflammation or of atopy. Factor analysis in our population based study also clearly reveals that frequently used objective parameters of asthma can behave independently. Subjects with symptoms do not always have airway obstruction and subjects with clear signs of airway inflammation can be completely asymptomatic. Instead of defining asthma as a simple disease, it seems to be more appropriate to discriminate different phenotypes of asthma.[38,39]

This concept of the existence of different asthma phenotype has important consequences for the interpretation of studies into the etiology of asthma, and is also important for clinical practice and treatment of patients in the future.[40]

In the current concept of asthma as a simple disease entity it has been postulated that both genetic and environmental factors play a role in the pathogenesis, probably by modulation of the immune system, resulting in pulmonary inflammation and disease symptoms. However, different genetic factors, in combination with different environmental factors, maybe affected by different time-frames might result in different asthma phenotypes. At the end it can not be excluded that some asthma phenotypes will be recognised as independent disease entities, or it may be that asthma comprises a continuous spectrum of partially overlapping phenotypes.

The concept of different asthma phenotypes might also explain the relatively disappointing results of studies into the hygiene hypothesis until now. If the endpoint of such studies is not one simple disease, but in fact is a combination of quite different disease entities, it is obvious why these studies do not result in uniform conclusions. It might explain why questionnaire-based studies come to other conclusions than studies which use for example lung function or IgE levels as an endpoint. It also explains why studies with a relative short follow-up have

different results compared to studies, like our own, with follow-up beyond childhood.

In future studies the borders of different asthma phenotypes should be discovered. Factor analysis can be an important tool to aggregate coherent phenomena in population based studies. Also in clinical practice it will be very important to elaborate different aspects of disease in all patients who get a diagnosis of “asthma”. In all patients all aspects of the disease (e.g. reported symptoms, lung function, bronchial hyper reactivity, airway inflammation and IgE levels) should be documented. Set-up of national and international prospective databases containing such extensive data in order to unravel different phenotypes can be a very promising development in the future.[41,42] To solve the question which childhood infections and other exposures may influence the development of atopic disease the hygiene hypothesis should be retested using these new endpoints. In this way different etiologic and also prognostic aspects of different asthma phenotypes might emerge.

Insight into etiologic differences between different asthma phenotypes can have important consequences for strategies with regard to prevention and treatment. So far, results of prevention studies using e.g. mite allergen reduction measures or vaccines are disappointing until now.[43,44] Recognition of the role of specific environmental exposures in the development of different asthma phenotypes will be necessary. Identification of such etiologic factors (or at least risk factors) might be helpful in better targeting of future preventive strategies.

Asthma phenotyping might also have great impact on current asthma treatment guidelines. Currently, the use of inhaled corticosteroids and bronchodilators is advocated in all patients with asthma. However, many recent studies have shown that with this current regimen only part of the patients with “asthma” can be controlled.[45] Adapting treatment protocols to different pathophysiologic mechanisms in different asthma phenotypes might be very helpful in increasing the rate of success.[40,46] Why should a patient without bronchial obstruction be treated with bronchodilators, and why should a patient with elevated IgE levels be treated with inhaled corticosteroids in stead of monoclonal anti-IgE antibodies? A relevant proportion of asthma patients is currently designated as having “brittle asthma” or as “difficult to treat asthma” by their clinicians. Especially these patients painfully show the current lack of knowledge about separate disease aspects and asthma phenotypes become visible.[46]

It is clear that we are still at the beginning of our knowledge about asthma. The future hides exiting and promising new results.

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Hoofdstuk 9

Samenvatting in het Nederlands

Walter Balemans

Samenvatting in het Nederlands

De verhoogde incidentie van astma en allergie in de laatste decennia wordt waarschijnlijk niet verklaard door genetische factoren maar door veranderende omgevingsinvloeden en de Westerse levensstijl. Voorbeelden hiervan zijn socio-economische factoren, veranderingen in eetgewoonten en verminderde expositie aan micro-organismen. Een evident voorbeeld hiervan is de toename van allergische ziekten in het voormalig Oost Duitsland na de val van het ijzeren gordijn. In een genetisch homogene bevolkingsgroep die ruim 40 jaar gescheiden had geleefd was vermoedelijk door uiteenlopende leefstijl een verschil in voorkomen van allergische ziekten ontstaan. Na de val van de muur werd het verschil tussen voormalig Oost en West in voorkomen van allergie snel kleiner.
In 1989 was het David Strachan die een nieuwe hypothese lanceerde, de zogenaamde ´hygiëne hypothese`. In een dwarsdoorsnede onderzoek in een groot Brits cohort constateerde hij een omgekeerde relatie tussen gezinsgrootte en de kans op het hebben van hooikoorts. Hij suggereerde dat in grote gezinnen de jongste kinderen vroeger werden blootgesteld aan allerlei infecties dan kinderen in kleine gezinnen en dat juist die vroege expositie aan microben zou beschermen tegen een allergische ontwikkeling. In de 90er jaren van de vorige eeuw kwam er steun voor deze hypothese vanuit immunologisch onderzoek bij proefdieren en bij mensen. De afweerrespons tegen virussen en bacteriën bleek een typisch patroon te hebben met zogenaamde T-helper 1 cellen (Th1). De T-helper 2 cellen (Th2), die betrokken zijn bij allergische reacties, bleken te worden onderdrukt door de Th1 respons. Meer recent werd duidelijk dat de kritische balans niet de Th1- versus Th2-status is, maar eerder een balans tussen T-regulatoire cellen en T-effector cellen die zowel de Th1- als de Th2- respons reguleren. T-regulatoire cellen blijken in de darm en het luchtwegepitheel te worden gestimuleerd via infecties en specifieke micro-organismen en induceren op die manier tolerantie en anti-inflammatoire reacties. Onvoldoende stimulatie van deze regulatoire cellen door micro-organismen zou kunnen leiden tot zowel auto-inflammatoire ziekten, zoals Diabetes type I en de ziekte van Crohn, als tot allergische ziekten zoals astma, hooikoorts en eczeem. (zie figuur 2.1 pagina 12)

Na de studie van Strachan verschenen er talloze epidemiologische studies over de hygiëne hypothese. De uitkomsten van deze studies zijn echter niet eenduidig en vooral de rol van luchtweginfecties in de ontwikkeling van allergie is onduidelijk. Het bewijs dat infecties op de jonge kinderleeftijd lijken te beschermen tegen astma en allergie is voornamelijk gebaseerd op studies die de relatie tussen gezinsgrootte en bezoek aan kinderdagverblijf en allergie later in het leven onderzochten. Gezinsgrootte en bezoek aan kinderdagverblijf zijn echter beiden indirecte markers voor infecties en een maat voor de algehele expositie aan infecties. Welke infecties of andere factoren in het vroege leven werkelijk een causaal verband houden met een verminderde kans op allergie en of luchtweginfecties hierin een sleutelrol in spelen is niet aangetoond. Integendeel, studies die naar surrogaat markers hebben gekeken vinden een beschermend effect, maar veel studies die direct de relatie tussen luchtweginfecties en allergie hebben bestudeerd vinden positieve associaties,

wijzend op een vergrote kans. In **hoofdstuk 2** wordt een uitgebreide samenvatting gegeven van de literatuur over de rol van infecties en luchtweginfecties in het bijzonder. Mogelijke redenen voor de genoemde paradox worden beschreven. De huidige aanwijzingen in het voordeel van de hygiëne hypothese berusten op observationeel onderzoek en talloze methodologische problemen, zoals allerlei vormen van bias, kunnen de resultaten van dit onderzoek beïnvloeden. Een voorbeeld hiervan is dat de tijdsrelatie tussen de infecties en de uitkomstmaat, zoals astma of allergie, vaak onduidelijk is. Het zou dus kunnen zijn dat een positieve relatie berust op omgekeerde causaliteit. Dat wil zeggen dat luchtweginfecties niet de oorzaak zijn van de allergische ontwikkeling, maar dat personen die een erfelijke aanleg hebben om allergisch te worden meer kans hebben op luchtweg infecties of meer symptomen hebben van luchtweginfecties. Voor dit laatste zijn de laatste jaren steeds meer aanwijzingen: allergische individuen hebben meer last van virale luchtweginfecties en meer lagere luchtwegklachten van doorgaans onschuldige virusinfecties. Een ander punt is dat de eindpunten of uitkomstmaten van verscheidene studies vaak sterk verschillen. Dit komt niet in de laatste plaats doordat er geen algemeen geaccepteerde definitie van astma bestaat. De opvolgtijd van verschillende studies wisselt ook sterk en er zijn maar weinig cohorten met een opvolgtijd tot na de kindertijd. Dit alles heeft sterk wisselende uitkomsten in de verschillende onderzoeken tot gevolg, die daarom moeilijk te vergelijken zijn.

Het cohort onderzoek van dit proefschrift bood een unieke kans om de rol van luchtweginfecties op de kinderleeftijd op de ontwikkeling van astma en allergie later in het leven te onderzoeken. Wij onderzochten een geboorte cohort uit het begin van de jaren tachtig. Ruim 1300 kinderen, geboren in Nijmegen tussen september 1982 en september 1983, waren tussen de leeftijd van 2 en 8 jaar prospectief onderzocht op het natuurlijk beloop van otitis media infecties. Nauwgezette en uitgebreide documentatie van bovenste luchtweginfecties en lagere luchtwegklachten werd verricht. Voor de huidige studie werden de deelnemers rond de leeftijd van 21 jaar opnieuw uitgenodigd deel te nemen. Er werd gebruik gemaakt van een vragenlijst naar het voorkomen van astma en allergische ziekten (de zogenaamde ISAAC Questionnaire werd gebruikt) en deelnemers werd gevraagd deel te nemen aan longfunctie onderzoek, meting van uitgeademd bronchiaal NO en bloedonderzoek naar allergie (IgE en Phadiatop bepaling). De resultaten van de studie staan beschreven in dit proefschrift.

In **hoofdstuk 3a** wordt de relatie beschreven tussen recidiverende luchtweginfecties op de kinderleeftijd en astma en allergische ziekten op jong volwassen leeftijd. De studie laat zien dat bovenste luchtweginfecties tussen 0 tot 2 jaar, 2 tot 4 jaar en 4 tot 8 jaar niet beschermen tegen astma, hooikoorts of eczeem op de leeftijd van 21 jaar. Lagere luchtwegklachten waren positief geassocieerd met astma bij jonge volwassenen. Deze relatie kan causaal zijn in beide richtingen, namelijk kinderen die lagere luchtweg infecties doormaken kunnen gevoelig zijn om vervolgens astma te ontwikkelen, of genetisch gepredisponeerde allergische kinderen kunnen meer gevoelig zijn om lagere luchtwegklachten te ontwikkelen bij virale luchtweg infecties. De laatst

genoemde hypothese wordt ondersteund door het feit dat kinderen van allergische ouders wat meer bovenste en onderste luchtweg infecties doormaakten. Dit gegeven wordt ook beschreven in andere studies, zoals in het Nederlandse PIAMA-cohort.
Onze studie is een van de weinige studies over de relatie tussen luchtweginfecties en allergie met een opvolgtijd van de kindertijd tot in volwassenheid. Het zou echter kunnen zijn dat vergelijk met studies in de kindertijd niet goed mogelijk is omdat volwassenen een ander fenotype astma zouden kunnen hebben dan kinderen. Aangezien wij niet over data beschikten betreffende allergie en astma op de kinderleeftijd, konden we het beloop van astma en allergie niet bestuderen. Bovendien, wordt het concept van de hygiëne hypothese niet alleen verklaard door het voorkomen van recidiverende bovenste luchtweg infecties. Expositie aan talloze andere factoren, zoals gastrointestinale infecties, en blootstelling aan gram-negatieve micro-organismen, kunnen het immuun stelsel beïnvloeden en de afweerrespons veranderen. Belangrijke veranderingen in de blootstelling aan deze factoren hebben de laatste decennia in de Westerse samenleving plaatsgevonden en deze kunnen verantwoordelijk zijn voor een shift van de afweerrespons in de richting van een Th2 – of allergische reactie.
Tot slot wordt door sommige auteurs wel gesuggereerd dat alleen infecties in het eerste levensjaar de ontwikkeling van allergie beïnvloeden. Andere studies echter laten weer zien dat ook exposities na het 3^{e} jaar nog invloed kunnen hebben op het ontwikkelen van hooikoorts klachten. Hoewel in onze studie de data betreffende infecties in de eerste twee jaren retrospectief werden verkregen, en daarmee wat minder valide kunnen zijn, konden wij geen relatie aantonen tussen bovenste luchtweginfecties en allergie voor en na de babyleeftijd.

Astma en allergie omvatten een heterogeen complex van kenmerken, zoals symptomen van piepen en benauwdheid, reversibele bronchusobstructie, luchtweg inflammatie, verhoogde bloedspiegels van IgE en overgevoeligheid voor specifieke allergenen. In veel studies is de uitkomst gebaseerd op vragenlijsten. Dit heeft tot gevolg dat de zo verkregen definitie van astma slechts ten dele de complexiteit van het ziektebeeld beschrijft. Daarom bestudeerden wij in **hoofdstuk 3b** de associatie tussen recidiverende luchtweginfecties op de kinderleeftijd en objectieve maten van astma en allergie op jong volwassen leeftijd. Bij 406 van de 693 deelnemende jongeren werd longfunctie, inclusief bepaling van reversibiliteit van bronchusobstructie, NO in uitgeademde lucht als maat voor luchtweg inflammatie en totaal IgE en specifiek IgE (met behulp van de Phadiatop) als maat voor allergische sensibilisatie gemeten. Recidiverend bovenste luchtweginfecties waren niet geassocieerd met longfunctie parameters, zoals de vitale capaciteit (FVC) en de 1 seconde waarde (FEV_1). Opvallend vonden wij een klein, doch significant, verschil in NO-waarde tussen individuen met recidiverende bovenste luchtweginfecties tussen de leeftijd van 0 tot 2. Kinderen die veel infecties doormaakten voor de leeftijd van 2 hadden een wat lagere NO-waarde, wat suggereert dat infecties zouden beschermen tegen allergische luchtweg inflammatie. Dit gegeven kon echter niet worden bevestigd door associaties met IgE of specifiek IgE, als maat voor allergie. Ook lagere luchtwegklachten in de

kindertijd waren niet geassocieerd met longfunctieparameters, reversibele brochusobstructie, NO en (specifiek) IgE op de leeftijd van 21 jaar. Deze uitkomsten bevestigen de resultaten uit **hoofdstuk 3a**, namelijk bovenste luchtweginfecties beschermen niet tegen allergie en astma bij jonge volwassenen, noch vergroten zij het risico hierop.

Adenotomie en tonsillectomie (ATE) (knippen van amandelen) is de meest uitgevoerde chirurgische ingreep bij kinderen met recidiverende bovenste luchtweg infecties. In **hoofdstuk 3c** bestudeerden wij de relatie tussen KNO ingrepen in de kindertijd en allergische ziekte op volwassen leeftijd. ATE kan gezien worden als surrogaat marker voor bovenste luchtweginfecties. Omdat bij deze ingreep functioneel lymfoid weefsel van het afweersysteem wordt verwijderd, is het denkbaar dat ATE de functie van het afweersysteem kan beïnvloeden. Verandering van de stimulatie van het afweersysteem kan op zijn beurt invloed hebben op de kans op het ontwikkelen van allergie. De resultaten van de studie lieten echter zien dat er geen associatie is tussen ATE en astma, hooikoorts en eczeem op de leeftijd van 21 jaar. Hoewel de inzichten de laatste jaren aan het veranderen zijn in de richting van meer terughoudendheid bij de indicatiestelling van deze ingreep bij jonge kinderen, konden wij geen aanwijzingen vinden voor schadelijkheid van ATE in termen van meer kans op allergie.

In **hoofdstuk 4** wordt een studie beschreven die werd verricht om onafhankelijke prognostische factoren op de kinderleeftijd te identificeren voor het ontwikkelen van astma op latere leeftijd. Veel studies beschrijven risico factoren voor het persisteren of recidiveren van astmatische klachten van de kindertijd tot in volwassenheid. Voorbeelden van bekende risicofactoren zijn: ernstig astma op de kinderleeftijd, het hebben van een andere allergische aandoening, allergie bij ouders, vrouwelijk geslacht, verminderde longfunctie en bronchiale hyperreactiviteit. Deze factoren geven echter geen inzicht in het individuele risico op astma van een bepaalde patiënt. Het vroegtijdig herkennen van risico groepen is belangrijk in termen van adequate behandeling en preventie. Er zijn nauwelijks studies gedaan met prognostische modellen voor astma. In de beschreven studie bepaalden wij prognostische factoren op basis van eenvoudige anamnestische gegevens bij kinderen van 0 tot 2 en 2 tot 4 jaar oud. Prognostische factoren tussen de leeftijd van 0 tot 4 jaar voor het hebben van astma op de leeftijd van 21 jaar waren vrouwelijk geslacht, roken door de moeder, lagere luchtwegklachten en allergie bij de ouder(s). Met deze onafhankelijke factoren werd een model opgesteld om de prognose voor een individuele deelnemer te kunnen bepalen. Het voorspellende model bleek echter matig te presteren en de kans op astma niet adequaat te kunnen voorspellen. De studie laat zien hoe een dergelijk voorspellend model kan worden opgesteld en worden vertaald in een predictiescore die te gebruiken is door de clinicus. Nieuwe, goed ontworpen prospectieve studies zijn nodig om voorspellende modellen te kunnen opstellen om de kans op het ontwikkelen van astma en allergie bij kinderen te kunnen voorspellen.

In **hoofdstuk 5** exploreerden wij de associaties tussen tussen uitgeademde NO-concentraties en allergische ziekte, IgE en longfunctie metingen. Bronchiaal

NO in uitgeademde lucht wordt gevormd door residentiele cellen in de luchtweg en wordt beschouwd als een maat voor allergische inflammatie van de luchtwegen. Het is eenvoudig, non-invasief te meten en is een veelbelovende parameter om de behandeling van astma met inhalatiesteroïden te monitoren. Voorgaande studies bestudeerden de rol van NO vooral in geselecteerde groepen van patiënten met astma. Ook werden reeds de determinanten van NO beschreven in de algemene bevolking bij kinderen en volwassenen. Over het algemeen namen deze studies echter niet alle allergische entiteiten in aanmerking. In onze studie bleken IgE, specifiek IgE, eczeem en hooikoorts onafhankelijke determinanten van verhoogd NO in uitgeademde lucht. Verrassend genoeg was NO niet onafhankelijk geassocieerd met astma. Hoewel NO waarden verhoogd bleken bij personen met astma plus allergie, verdween het effect na correctie voor voor de overige factoren.
De onafhankelijke invloed van hooikoorts en eczeem op NO-waarden in onze ongeselecteerde populatie suggereert dat de verhoogde waarden bij individuen met astma, zoals gevonden in andere studies, ook verklaard kan worden door het samengaan van astma met andere allergische aandoeningen zoals eczeem en hooikoorts. Dit fenomeen wijst op een gemeenschappelijk onderliggend immunologisch mechanisme voor de verscheidene allergische aandoeningen.

In **hoofdstuk 6** bestudeerden we het voortduren van recidiverende bovenste luchtweginfecties van de kindertijd tot in volwassenheid en de geassocieerde medische consumptie. Tevens bekeken we of recidiverende bovenste luchtweginfecties in de vroege kinderjaren een risico factor vormen voor deze infecties later in het leven. Van de onderzochte studiepopulatie bleek 23% te lijden aan terugkerende periodes van bovenste luchtweginfecties, terwijl slechts 1% leed aan het persisteren van bovenste luchtweginfecties gedurende de kindertijd tot op volwassen leeftijd. Ruim 30% van de deelnemers had meermaals antibiotica gebruikt voor bovenste luchtweginfecties tussen 0 en 21 jaar en 32% had een KNO ingreep ondergaan. Van de 166 deelnemers (24%) die tussen 8 en 21 jaar recidiverende bovenste luchtweginfecties doormaakten, had 84% een eerdere episode van recidiverende bovenste luchtweginfecties doorgemaakt.
Deze resultaten laten zien dat bovenste luchtweginfecties zeer veel voorkomen gedurende vooral de kindertijd en bij ongeveer een kwart van de deelnemers ook op jong volwassen leeftijd. Het is daarom een uitdaging om strategieën te ontwikkelen om recidiverende bovenste luchtweginfecties te voorkomen, bijvoorbeeld met preventieve vaccinatieprogramma's.

In **hoofdstuk 7** onderzochten we of domeinen, samengesteld uit op vragenlijst gebaseerde items waren geassocieerd met domeinen bestaande uit objectieve maten van astma en allergie, zoals longfunctie parameters (FEV_1, FVC, reversibiliteit van bronchiale obstructie), (specifiek) IgE en uitgeademd bronchiaal NO. Met een specialistische statistische methode, zogenaamde *factor analyse*, konden zowel de verscheidene subjectieve items uit de vragenlijst als de objectieve metingen betreffende astma en allergie worden geclusterd in een aantal min of meer onafhankelijke domeinen, of zogenaamde *factoren*. De subjectieve factoren bestonden uit: ' astma symptomen', 'dokters

diagnose astma', 'hooikoorts', en 'allergische ziekte bij ouder(s)'. Objectieve factoren waren: 'longfunctie' en 'IgE, specifiek IgE en NO'. Wij lieten in deze studie zien dat de subjectieve factoren nauwelijks relatie hadden met de objectieve factoren. Het benadrukt de heterogeniteit van astma en laat bovendien de beperkte relatie zien tussen de verscheidene onderdelen van het symptomen complex, zoals klachten, longfunctie en allergische inflammatie. Deze studie sluit aan bij een nieuw concept dat astma en allergie uit verschillende onafhankelijke entiteiten bestaan die slechts gedeeltelijk overlappen, met ieder hun eigen pathofysiologisch mechanisme.

Discussie en aanbevelingen

De studies die worden gepresenteerd in dit proefschrift stellen de huidige astmaconcepten ter discussie en hebben belangrijke consequenties voor toekomstig onderzoek naar de etiologie en pathogenese van de ziekte.
Ons onderzoek toont duidelijk dat astma geen eenvoudige en eenduidige ziekte is. Hoewel in veel studies astma wordt vereenvoudigd tot een enkel symptoom, zoals 'piepen in de laatste 12 maanden', laat dit proefschrift zien dat subjectieve astma symptomen niet of nauwelijks zijn geassocieerd met objectieve parameters, zoals luchtwegobstructie, luchtweg inflammatie en allergie. De verrichte *factor analyse* bevestigde ook dat de gemeten objectieve parameters zich onafhankelijk gedragen. Individuen met klachten van benauwdheid hebben niet altijd aantoonbare luchtwegobstructie en individuen die geen symptomen hebben kunnen evidente tekenen van luchtweg inflammatie hebben. In plaats van astma te definiëren als een eenvoudig ziektebeeld lijkt het dus reëler om verschillende fenotypen van astma te onderscheiden. Het concept van verschillende typen van astma heeft belangrijke gevolgen voor de interpretatie van etiologische studies en zal in de toekomst ook zijn weerslag hebben in de klinische praktijk en behandeling van astma. In het huidige eenvoudige astmaconcept wordt aangevoerd dat zowel erfelijke als omgevingsinvloeden een belangrijke rol spelen bij het ontstaan, waarschijnlijk door modulering van het afweersysteem, resulterend in inflammatie in de long en symptomen bij de patiënt. Echter, verschillende genetische factoren, in combinatie met verschillende omgevingsfactoren die zich voordoen in verschillende leeftijdsfasen zullen resulteren in verschillende astma fenotypes. Uiteindelijk kan niet worden uitgesloten dat een aantal van deze astma fenotypes als onafhankelijke ziekte-entiteiten zullen worden herkend. Het kan ook zijn dat blijkt dat astma meer een continu spectrum is van elkaar deels overlappende fenotypes.
Het concept van verschillende onafhankelijke astma fenotypes kan een goede verklaring zijn voor de tot dusverre tegenstrijdige en teleurstellende resultaten van studies over de hygiëne hypothese. Als het eindpunt van deze studies niet een eenvoudig eenduidig ziektebeeld betreft, maar een heel scala aan ziekte-entiteiten, is het duidelijk dat deze studies niet tot eenduidige conclusies komen. Het verklaart waarom op vragenlijsten gebaseerde onderzoeken tot andere conclusies komen, dan studies die gebruik maken van long functie of IgE als eindpunt. Het verklaart tevens waarom studies met een relatief korte follow-up andere resultaten vinden dan studies met een follow-up tot na de kindertijd, zoals ons eigen onderzoek.

In toekomstig onderzoek zullen de grenzen van de verscheidene astma types moeten worden onderzocht. De door ons gebruikte factor analyse kan daarbij een uitstekend statistisch instrument zijn om bepaalde coherente fenomenen in populatie studies te clusteren. Ook in de klinische praktijk zal het steeds belangrijker worden om de verschillende aspecten van de ziekte van een patiënt met de diagnose ‘astma’ te bekijken, zoals gerapporteerde symptomen, long functie, bronchiale hyperreactiviteit, luchtweg inflammatie en IgE waarden. Het opzetten van nationale en internationale databanken die uitgebreide informatie bevatten omtrent al deze ziekteaspecten, met als doel het verder ontrafelen van de verschillende fenotypes, is een veelbelovende ontwikkeling. Om de vraag te beantwoorden welke infecties in de kindertijd en andere omgevingsinvloeden de ontwikkeling van astma en allergie beïnvloeden zullen studies moeten worden verricht naar de verschillende aspecten van de hygiëne hypothese, die gebruik maken van nieuwe op verschillende fenotypes gebaseerde eindpunten. Zo kunnen de verschillende etiologische en prognostische aspecten van de verschillende astma types naar voren komen. Inzicht in etiologische verschillen tussen verschillende astma fenotypes kunnen belangrijke consequenties hebben voor preventie- en behandelingsstrategieën. Tot dusverre waren de resultaten van studies naar primaire preventieve maatregelen, zoals bijvoorbeeld huismijt reductie, teleurstellend. Het herkennen van de rol van specifieke omgevingsfactoren in de ontwikkeling van verschillende astma fenotypes is van belang om in de toekomst preventieve maatregelen beter te kunnen inzetten.

Het fenotyperen van astma zou ook grote gevolgen kunnen hebben voor de huidige behandelingsrichtlijnen van astma. Volgens de huidige richtlijn worden luchtwegverwijders en inhalatiesteroïden bepleit voor alle astma patiënten. Recente studies laten zien dat volgens dit regime slechts een gedeelte van de patiënten met astma voldoende controle hebben van hun klachten. Het aanpassen van de richtlijnen op basis van verschillende pathofysiologische mechanismen bij verschillende astma fenotypes zou de behandelresultaten kunnen verbeteren. Waarom zouden patiënten zonder evidente bronchusobstructie worden behandeld met bronchusverwijders en waarom zou een patiënt met verhoogd IgE worden behandeld met inhalatiesteroïden in plaats van met monoklonale anti-IgE antistoffen? Een relevant aandeel van de huidige patiënten met astma wordt door de behandelaren bestempeld als ‘moeilijk behandelbaar astma’. Juist in deze groep wordt pijnlijk duidelijk hoe weinig we weten van de onafhankelijke ziekteaspecten en de verschillende astma fenotypes. We staan pas aan het begin van de kennis van de aspecten van dit heterogene ziektebeeld. De toekomst verbergt nog uitdagende en veelbelovende kennis.

Curriculum Vitae

List of Publications

Walter Balemans

Curriculum Vitae

Walter Balemans werd geboren op 4 november 1967 te Zwijndrecht. Hij volgde zijn middelbare schoolopleiding aan het Christelijk Lyceum te Zeist, waar hij in 1986 het diploma Atheneum B behaalde. In dat jaar begon hij met de geneeskunde studie aan de Universiteit van Utrecht. Hij was daar actief in het studentenleven, onder andere als Praeses van de Medische Studenten Faculteits Vereniging Utrecht “Sams” en als Praesident van het Koninklijk Utrechtsch Studenten Tooneel. In 1993 behaalde hij het doctoraal examen en deed in dezelfde periode onderzoek naar spierschade als gevolg van excentrische training in een diermodel op het laboratorium voor experimentele neurologie onder leiding van Prof. Dr. P.R. Bär. In 1995 behaalde hij het artsexamen. In datzelfde jaar trouwde hij met Margreet Vink waarmee hij inmiddels 6 kinderen heeft. Vanaf september 1995 werkte hij gedurende een jaar als AGNIO op de afdeling kinder- en jeugdpsychiatrie van de psychiatrische instelling Veldwijk in Ermelo. In september 1996 begon hij met de opleiding Kindergeneeskunde in het Wilhelmina Kinderziekenhuis Utrecht, aanvankelijk onder opleiderschap van Prof. Dr. A. Okken en later Prof. Dr. J.L.L. Kimpen. Tussen januari 1998 en januari 2000 was hij arts-assistent bij de vakgroep kindergeneeskunde van het Catharina Ziekenhuis onder opleiderschap van Dr. J. Waelkens. In 2001 volgde de registratie tot kinderarts en werd aangevangen met het fellowship bij de afdeling kinderlongziekten van het UMC in het Wilhelmina Kinderziekenhuis onder leiding van Dr. C.K. van der Ent. Onder diens inspirerend opleiderschap werd in 2003 een aanvang gemaakt met het onderzoek dat thans heeft geleid tot dit proefschrift. Per 1 december 2005 is hij geregistreerd kinderlongarts en per 1 januari 2006 werkt hij als kinderarts en waarnemend opleider bij de vakgroep kindergeneeskunde van het St. Antonius Ziekenhuis in Nieuwegein.

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