# VIRAL RESPIRATORY INFECTIONS IN PATIENTS WITH CHRONIC NON-SPECIFIC LUNG DISEASE (CNSLD)

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The cover illustration is a graphic composition of the building of the Netherlands Asthma Centre in Davos and the model of an influenza virus.

# VIRAL RESPIRATORY INFECTIONS IN PATIENTS WITH CHRONIC NON-SPECIFIC LUNG DISEASE (CNSLD)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE
GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. J. SPERNA WEILAND
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 30 SEPTEMBER 1981 DES NAMIDDAGS
TE 2.00 UUR

DOOR

ALBERT CORNELIS ROLDAAN

GEBOREN TE ROTTERDAM

1981
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The survey described in this thesis was carried out in the Netherlands Asthma Centre in Davos, Switzerland, and in the Department of Virology, Erasmus University Rotterdam. Financial support was received from the Netherlands Asthma Fund (Project no. 275) and the "Vereniging Nederland-Davos".

*Voor Marion, Tim en Karine,*
*mijn ouders,*
*Paul Zuidema*

*Wie is de gelukkige mens?*
*Die andermans verdienste*
*weet te waarderen en die*
*zich in het genot van de ander*
*verheugt als betrof het hemzelf.*

*Goethe*

CONTENTS

## GENERAL INTRODUCTION

It is a well known clinical phenomenon that patients suffering from bronchial obstructive disorders, usually covered by the term chronic non-specific lung disease (CNSLD) tend to react with exacerbations of their disease in the course of respiratory infections, especially if a viral or mycoplasmal agent is involved. However, there is no uniform opinion about the relative importance of viral respiratory infections in this respect, and much of the background of the features observed in virus-induced exacerbations is still unknown. This is at least partly due to semantic uncertainty with regard to both respiratory infection and the various clinical entities encompassed by the spectrum of CNSLD. The term CNSLD covers conditions most commonly referred to as bronchial asthma, asthmatic bronchitis, chronic bronchitis, and pulmonary emphysema.

The Netherlands Asthma Centre in Davos, Switzerland, is a clinic for treatment of Dutch CNSLD patients. It is situated 1560 m above sea level, where climatic conditions have been shown to affect the course of the bronchial obstructive disease in a favourable way. Patients are usually referred to the clinic for a period of at least several months.

In 1975 the suggestion was put forward that the situation in the Davos clinic was highly suitable to investigate some aspects of the above mentioned relationship between respiratory viral infection and flare-ups of signs and symptoms of CNSLD. A 4-year study was designed in order to find an answer to the following questions:

1. Which is the frequency of symptomatic respiratory infection (SRI) in a group of CNSLD patients during their stay in an alpine climate?
2. In how many episodes of SRI can a viral or mycoplasmal agent be identified as a causative factor, doing systemically conducted serological investigations?
3. To which extent do respiratory infections of known and unknown origin cause exacerbations of CNSLD?

4. Are there any differences in infection rate and/or reaction pattern to respiratory infection between various subpopulations of CNSLD patients?
5. Are there any differences in reaction pattern with regard to causative agent and clinical severity of respiratory infection?
6. How often does bacterial infection occur in the course of SRI?

In the second part of this thesis the design of the study and the results are presented. The first part contains a number of chapters dealing with general information with regard to CNSLD (chapter 1) and respiratory virology (chapter 2), as well as comments on the role of viral respiratory infection in the cause (chapter 3) and course (chapter 4) of CNSLD. Furthermore, a short review on climatic factors in CNSLD (chapter 5) and treatment in the Netherlands Asthma Centre (chapter 6) is presented in this part.

# PART I

# CHAPTER 1
# CHRONIC NON-SPECIFIC LUNG DISEASE (CNSLD)
# A REVIEW ON TERMINOLOGY

## 1-1 Introduction

In 1965 a Lancet editorial[152] commented that "few diseases have been surrounded by such diagnostic confusion as those whose clinical features are cough, sputum and breathlessness with wheezing, which are variously described as asthma, chronic bronchitis or emphysema".

Indeed, terms like bronchial asthma, asthmatic bronchitis, chronic bronchitis and pulmonary emphysema have led to much confusion. A few decades ago the term chronic bronchitis was used in Britain to describe the same condition that was generally called emphysema in North America, as was pointed out in a study by Fletcher *et al.*[76]

The ambiguity that existed was a serious obstacle in understanding the conditions which are attended with chronic obstruction of airflow. It was primarily for this reason that a group of British physicians met at a CIBA Foundation Guest Symposium and produced a set of definitions in 1959.[44] The committee coined the term Chronic Non-Specific Lung Disease (CNSLD) to encompass the conditions usually termed asthma, chronic bronchitis and emphysema. (In The Netherlands the term was introduced as CARA = "Chronische Aspecifieke Respiratoire Aandoeningen"). The conclusions drawn at the symposium were amended by the World Health Organization[297] and the American Thoracic Society.[4] The idea of using an all-over term was also sustained at the International Bronchitis Symposia held in Groningen in 1961, 1964, and 1969.[60 74 205]

Sluiter[241] summarized the various arguments for the use of a general term to cover the three conditions already mentioned:

-There is no well defined separation between the syndromes, usually called asthma, chronic bronchitis, and emphysema, but a fluent transition.

-Symptomatology of the different conditions is likely to change in the

course of life, thus enabling the use of various diagnoses for the same underlying disease.

-There is no possibility to describe the seriousness of the symptoms in a quantitative way, using terms like asthma, chronic bronchitis and emphysema only.

Furthermore, Doeleman[59] suggested that data regarding the family histories of patients with these conditions are confusing and in no way helpful in differentiating between them.

Patients suffering from chronic non-specific lung disease may be defined as subjects with one or more of the following symptoms: chronic or recurrent cough with expectoration and/or persistent excessive breathlessness, which are not solely attributable to other diseases:

a. Localized lung disease of any kind (e.g. tuberculosis, pneumonia, bronchiectasis, cystic disease).
b. Generalized specific infective lung disease (e.g. miliary tuberculosis).
c. The pneumoconioses.
d. Collagen disease and the generalized pulmonary fibroses and granulomata.
e. Primary cardiovascular-renal disease.
f. Disease of the chest-wall.
g. Disease of the upper respiratory tract.
h. Psychoneurosis.

For epidemiological purposes the words chronic or recurrent may be defined as occurring on most days for a least three months in the year during at least two consecutive years.

1-2 The Dutch point of view

In The Netherlands the prevailing opinion is that the large majority of patients with CNSLD are characterized by an increased bronchial reactivity to various stimuli, which results in a (partially) reversible diffuse bronchial obstruction. This opinion was particularly put forward by the Groningen workgroup of Orie and associates who stated that there does not seem to be a fundamental difference between the clinical entities of CNSLD: asthma, bronchitis and emphysema. It was their point of

view that it is preferable to avoid the use of these names and to stress the necessity of an exact description of each individual patient. They suggested that in each case of CNSLD, apart from the clinical symptoms, the following characteristics should be established:[203 241]

-Bronchial hyperreactivity and its degree.
-Allergy and its degree.
-Bronchial obstruction and its degree.
-Degree of reversibility of bronchial obstruction.
-Presence of complicating factors, especially bacterial bronchial inflammation.
-Sputum eosinophilia and its degree.
-Presence of additional pulmonary disease.
-Supplementary data, like $\alpha_1$-antitrypsin content of the serum.

Generalized bronchial obstruction is considered to be the major pathophysiological sequence in patients with CNSLD. The clinical picture is determined by various endogenous and exogenous factors. Fig. I-1 shows a summary of these factors according to Van der Lende *et al.*[157] The importance of these various factors was discussed in detail by Van der Lende[156] and Westermann.[292] It has been suggested that there is a relationship between CNSLD and endocrine dysfunction.[124 289 293]

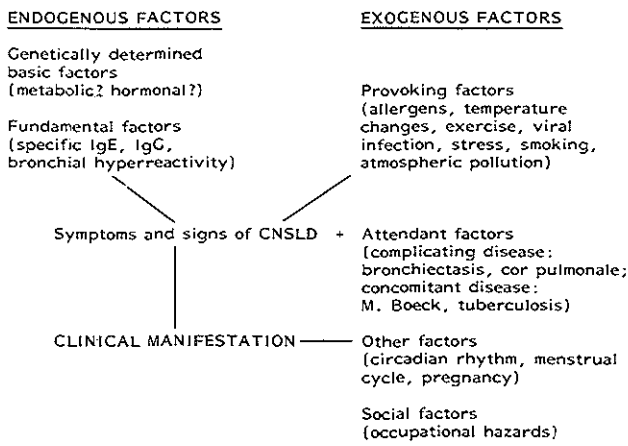


Fig. I-1 The role of various factors in the development of CNSLD, according to the hypothesis of the Groningen Work Group[156] (slighly modified)

Bronchial hyperreactivity is an important factor in patients with CNSLD and can be defined as the symptom of an increased reactivity to exogenous non-allergic substances. The threshold to inhaled histamine reflects the responsiveness of the bronchial system[284 285], whereas the potency to develop reagins is often used to determine the activity of allergic factors.[139]

Gökemeijer[91] suggested that different profiles in reactivity can be found in different groups of patients with CNSLD and that the degree of hyperreactivity is an important co-determinating factor in the quantitative response during allergen provocation. This suggestion is strongly supported by data from several investigators.[46 198 200] There seems to be a small effect on the degree of hyperreactivity after allergen inhalation as well.

1-3 The Anglo-Saxon point of view

Although the proposals made at the CIBA Guest Symposium[44] gained wide acceptance, some confusion remained.[73] Though the need for an encompassing term was generally accepted, some investigators denied the benefit of grouping conditions like asthma, bronchitis, and emphysema.[120 216] Various reports on the subject appeared: Medical Research Council Committee[177], CIBA Foundation Study Group[43], ACCP-ATS Joint Committee on Pulmonary Nomenclature.[223]

Except for The Netherlands, where the term CARA is widely accepted, the originally proposed term CNSLD has somewhat fallen into disuse. The main reasons for this are that the term is cumbersome in English, refers only to symptomatic patients and excludes bronchiectasis.[270] It has been progressively replaced in Anglo-Saxon literature by terms such as chronic obstructive lung disease (COLD), chronic obstructive pulmonary disease (COPD), chronic obstructive airway disease (COAD), and chronic airflow obstruction (CAO). These terms, however, have problems of their own and semantic uncertainty still remains.

The proposal to avoid the terms asthma, chronic bronchitis, and emphysema, whenever possible, has never been generally accepted, probably because of disagreement with regard to the hypothetical common fundamental aetiology of these conditions. Throughout the literature these

diagnoses are widely used and therefore a short review of the most frequently used definitions is necessary. A concise comment on bronchiectasis will be given as well.

1-3-1 Bronchial asthma

Asthma is usually described as a disease characterized by an increased responsiveness of the trachea and bronchi to various stimuli and manifested by a widespread narrowing of the airways that changes in severity either spontaneously or as a result of therapy.[4]

Scadding[235] gave the following definition: "Asthma is a disease, characterized by wide variations over short periods of time in resistance to flow in the airways of the lungs" and proceeded with some explanatory comments.

Antigen-antibody reactions play an important role in a proportional part of asthmatic patients. The external source of the causal factor is usually indicated by the word extrinsic. Those allergic patients who produce IgE antibodies can be categorized as having extrinsic atopic asthma, whereas subjects who are not atopic and in whom asthma can be shown to be caused by reactions between environmental antigens and antibodies of other types than IgE[214], are usually classified as suffering from extrinsic nonatopic asthma.

Allergic asthmatic reactions can be divided into three main groups: immediate, nonimmediate, and late, and dual or combined reactions in which both immediate and late reactions occur. There are at least two forms of immediate and three forms of late asthmatic reactions, according to Pepys.[215]

Immediate reactions usually result from type I (IgE mediated) allergic mechanisms, leading to mast cell degranulation and release of mediators, thus causing bronchial obstruction. It is now known that these chemical mediators of immediate hypersensitivity can be divided into preformed (primary) substances like histamine, serotonin, eosinophil chemotactic factor of anaphylaxis (ECF-A), intermediate molecular weight ECF(s), neutrophil chemotactic factor, heparin, chymase, N-acetyl-β -glucosaminidase and arylsulfatase A, and unstored (secondary) mediators like slow reacting substance of anaphylaxis (SRS-A), platelet activating

factors (PAFs), and lipid chemotactic and chemokinetic factor.[9]

The mechanisms of late reactions are still incompletely understood. Type III precipitating antibodies are supposed to play a role in the reaction which is maximal at about 5-8 hours after allergen provocation.[161 162] Pepys[217] suggested that the immediate reaction, even if it remains clinically undetected, induces increased vascular permeability, so that immune complexes can precipitate on endothelial membranes.

To those patients who have well marked asthma, though none of the immunological reactions can be detected, the term "intrinsic" asthma is usually applied. Some authors prefer the word "cryptogenic" asthma[278] because it implies that provocative agents are unknown at the present time. Typically, this type of asthma starts in middle or later adult life, shows marked eosinophilia of blood and sputum and tends to respond poorly to bronchodilating drugs, but promptly to corticosteroids. The family histories of these patients seem to show fewer members with hayfever and eczema, but almost identical figures with regard to asthma as compared to patients with extrinsic asthma.[277]

A disturbance in the regulation of bronchial smooth muscle tone seems to be a basic defect in bronchial asthma. Szentivanyi[264] presented a hypothesis that a partial β-adrenergic blockade constitutes the fundamental factor for this dysregulation. On the other hand, it has been shown that parasympathetic mechanisms also play an important role in controlling bronchial smooth muscle tone[92 93 196] and that some anticholinergic drugs, like thiazinamium, have potent bronchodilating properties.[18]

### 1-3-2 Chronic bronchitis

The term chronic bronchitis is most commonly defined as the condition of subjects with chronic or recurrent excessive mucous secretion in the bronchial tree.[44] This description is based on clinical criteria, in contrast to the definition of asthma, in which functional characteristics (resistance to flow) are often used.

Chronic bronchitis often occurs in combination with pulmonary emphysema. It is estimated that about 30% of chronic bronchitics have no emphysema, whereas 80% of patients with emphysema have chronic bronchi-

tis as well.[187 272 273 300]

For generations, British physicians have used the term chronic bronchitis synonymously with the syndrome of chronic airflow obstruction and, unfortunately, some still do. So the term was used without qualification to indicate, at one extreme, regular production of small quantities of sputum without any functional abnormality and, at the other extreme, severe chronic airflow obstruction.

The use of a single term to cover such a wide range of abnormalities was justified by the hypothesis that they embraced the natural course of a single disease and that a mere productive cough was regarded as its earliest stage.[75 186] Epidemiological investigations by Fletcher and associates[73], commented in The Lancet[22], suggest that mucous hypersecretion, bronchial infection, and airflow obstruction consist of two largely distinct processes, caused by two linked susceptibilities to smoking and other forms of air pollution. On the one hand, there is mucous hypersecretion, which increases liability to recurrent bronchial infection and, on the other hand, there is the development of bronchial obstruction.

The first, the hypersecretory disorder, consists of hypersecretion of bronchial mucus, derived from enlarged bronchial mucus glands and hyperplastic goblet cells, producing a productive cough. It is considered as reaction of susceptible individuals to smoking and to other forms of air pollution.

The latter, the obstructive disorder, is due to disease of the airways and to emphysema. Its main cause is also cigarette smoking with general and industrial air pollution as contributory causes.

Because of the common causes and because these disorders develop only in subjects with constitutional susceptibilities, which are often linked with each other, they commonly occur together, but they may develop independently of each other.

The obstruction to airflow found in patients with the obstructive disorder is thought to be distinct from asthma and to have a largely irreversible character. Whether the patients secrete too much mucus or not and whether or not they have repeated bronchopulmonary infections does not affect the outcome. The prognosis of these patients is deter-

mined mainly by the degree to which expiratory flow is impaired.[135] Besides, the degree of reversibility after administration of thiazinamium, a bronchodilator, and the rate of decrease in initial $FEV_1$ per year have been shown to have important prognostic significance.[221]

On the base of their findings Fletcher *et al.* proposed to abandon the term chronic bronchitis and replace it by chronic mucous hypersecretion and chronic airflow obstruction to describe the above mentioned disorders. The suggestion was adopted by Thurlbeck.[271]

1-3-3 Pulmonary emphysema

Pulmonary emphysema is usually defined as an increase in the size of air spaces, distal to the terminal bronchioles either from dilatation or from destruction of their walls.[44] This definition is couched in anatomic terms and therefore it is inaccurate to use the term emphysema in living subjects, although clinical and pathological correlative studies by Thurlbeck *et al.*[273] suggest an association of the clinical and functional syndrome or syndromes with the disordered anatomy.

Much of the disability occurring in advanced emphysema is probably due to associated airway lesions, causing progressive chronic airflow obstruction, which is the functional characteristic of the disease.

Dornhorst[61] is generally credited with the observation that patients with chronic airflow obstruction may present with one or two very different clinical syndromes. One syndrome is characterized by hypoxemia and hypercapnia accompanied by oedema, thought to be due to marked right ventricular failure (type B = blue bloater). In the other syndrome right ventricular failure is absent, blood gases are relatively well maintained, and minute ventilation is greatly increased (type A = pink puffer). The blue bloater is often referred to as chronic bronchitis and the pink puffer as emphysema, which is at best an oversimplification. Although all agree that the syndromes exist, the exact definitions of the conditions and their underlying pathology are in dispute. For practical purposes the classification is of little use, as the majority of patients with chronic airflow obstruction cannot be categorized in one of the two syndromes.[55]

Emphysema has been classified on the basis of the location of the

lesions in the secondary lung lobule into:

a. Centrilobular emphysema, involving initially the first-order respiratory bronchioles.
b. Panlobular emphysema, with diffuse involvement of the respiratory air spaces.
c. Paraseptal or subpleural emphysema, in which the respiratory air spaces adjacent to fibrous septa are first involved.

Centrilobular emphysema frequently gives rise to increased thickening and frequency of bronchovascular markings on the chest röntgenogram (increased marking emphysema), whereas the panlobular type is often associated with peripheral vascular deficiency (arterial deficiency emphysema).[273]

The pathogenesis of emphysema is not completely understood. The subject has recently been discussed in detail by Hugh-Jones and Whimster[120] and by Franken.[78] Since there are several forms of emphysema, a variety of aetiologic and pathogenetic factors may be involved rather than just one. Endogenous factors leading to increased susceptibility certainly play a role in the development of pulmonary emphysema. Hyperreactivity is a feature not only found in asthmatics but also in patients with largely irreversible airflow obstruction.[91 145 240] Allergy also seems to be important in this respect.[129 230 303] It is an interesting issue that the obstructive changes have a progressive character, although hyperreactive and allergic features tend to diminish, according to Orie.[202 204]

The occurrence of severe pulmonary emphysema in association with $\alpha_1$-antitrypsin deficiency, described by Laurell and Eriksson[151], together with the observation that papain and elastase[99 132 137] produce emphysema in experimental animals, stressed the importance of proteolysis in human emphysema.

Cigarette smoking is considered to be the most important factor in the development of emphysema.[8 73] Current theories hold proteinases from polymorphonuclear leucocytes sequestered in the pulmonary capillaires or from alveolar macrophages responsible for the damage to the connective tissue in the lung[212], but the literature does not provide conclusive evidence.[78]

1-3-4 Bronchiectasis

Bronchiectasis refers to a condition of the lung in which there is permanent, abnormal dilatation of the bronchi.[273] It may be classified on anatomic grounds into cylindrical, varicose or saccular bronchiectasis, depending on the appearance of the dilated bronchi.[229]

Many patients characteristically date symptoms from an acute infective episode, usually in infancy or childhood.[83 260 261] These patients are often referred as having postinfective bronchiectasis. Bronchiectasis will often be associated with symptoms similar to those found in patients with CNSLD, but is by definition not comprised in the spectrum of CNSLD, unless symptoms are not solely attributable to the bronchiectatic lesions. Nevertheless, it seems worthwhile to pay attention to this condition, because it is often ignored as a cause of chronic obstructive lung disease. Moreover, it is important to stress that bronchiectasis may complicate asthma[62], bronchitis and emphysema[63 273] and is often related to allergy.[288]

1-4 Conclusions

On reviewing the literature one must conclude that international uniformity of nomenclature has not yet been achieved and probably will not be realised for some time. Therefore, it seems necessary to describe patients with clinical features like cough, phlegm, and breathlessness with wheezing as precisely as possible, using the characteristics listed in chapter 1-2. The use of an encompassing term is recommendable, provided that this does not inhibit attempts to search for fundamental factors, distinguishing between the various conditions, described in this chapter.

In this thesis the following guiding principles will be employed in the classification of patients:

a. Preference is given to the omnibus-term chronic non-specific lung disease(CNSLD), mainly because its synonym CARA is generally accepted in The Netherlands and the term includes asthma.
b. The term chronic airflow obstruction (CAO), which has much to recommend for, will be used to describe the patients suffering from progressive, largely irreversible obstructive disease, due to

conditions like emphysema.

c. On account of the data presented in chapter 1-3-2, the use of the name chronic bronchitis will be avoided. This term will only be employed with reference to the literature on the role of infections in CNSLD, where its use is inevitable.

d. The condition of those persons merely suffering from the hypersecretory disorder -only very rarely seen in a clinic for CNSLD- will be called chronic mucous hypersecretion.

e. The presence of suspected or proven bronchiectatic lesions will be mentioned seperately.

# CHAPTER 2
# RESPIRATORY VIRAL INFECTIONS IN MAN

## 2-1 Introduction

Within the past decades many new viruses have been isolated and associated with respiratory illness in humans. In this chapter a short outline of the respiratory viruses which have been studied in the survey, described in this thesis, will be presented. Short comments on the non-viral agents *Mycoplasma pneumoniae* and *Chlamydia psittaci* will be given as well.

## 2-2 Adenovirus

### 2-2-1 Introduction

Adenoviruses derive their name from the fact that they were first isolated from adenoid tissues (tonsils) and have a certain affinity for lymph glands. They also invade the respiratory tract, the gastrointestinal tract, and the conjunctiva. In the respiratory tract they may cause a variety of clinical manifestations, ranging from pharyngitis to bronchitis, croup, and pneumonia. Adenovirus infections are widely distributed and common. Most infections occur in childhood and remain subclinical. About adenoviruses much detailed structure and biochemistry are known. Inactivated and live vaccines have been developed and found effective.

### 2-2-2 Characteristics

Adenoviruses are double-stranded DNA viruses lacking an envelope.[126 201] The diameter of the virion has been measured as 60-90 nm.[106 117] The capsid is made up of 252 hollow capsomeres arranged in an icosahedral symmetry. Adenoviruses are recognized by a common group antigen in a complement fixation test. By haemagglutination the 31 immunologically distinct types causing human infection can be determined. The replication cyclus requires at least 30 hours.

Adenoviruses are unusually stable to physical and chemical agents and adverse pH conditions resulting in prolonged survival outside the host cells and great potential for spread.

2-2-3 Laboratory methods

Adenoviruses are propagated most readily in continuous cell lines of human epithelial cells, such as HEK, Hep-2, and HeLa cells.[49] [126] Properly interpreted virus isolation provides the most conclusive evidence of infection and offers the opportunity to determine the serotypes using a neutralization test with type-specific rabbit antisera. The three commonly applied serological tests are the complement fixation (CF), neutralization, and haemagglutination inhibition tests.[126] The CF test measures group-specific antibody, is the easiest to perform and is most useful in diagnosis of acute infections, since CF antibodies tend to disappear rather rapidly. Haemagglutination inhibition and neutralization tests are used in serological epidemiology.

2-2-4 Association with human illness

Of the numerous clinical syndromes that have been associated with adenovirus infections, some have been proven to be causally related, whereas the evidence for specific aetiology for other syndromes remains in question. A few types of adenovirus are associated with a single clinical syndrome, but the majority cause different syndromes in diversified populations. The disease syndromes attributed to adenovirus include:

-Undifferentiated acute respiratory disease.
Types 4 and 7 are the major agents in military populations, whereas infections have also been observed in civilian adults. Clinical signs are sore throat, often with cervical lymphadenopathy, cough, chills, fever, malaise, headache, and sometimes rash. The incubation period is 5-6 days, and the onset is usually gradual.

-Pharyngoconjunctival fever.
Types 3 and 7 are the main cause of this syndrome, characterized by the triad of conjunctivitis, pharyngitis, and fever.

-Pharyngitis.
Febrile pharyngitis without conjunctivitis, caused by types 3,4,7,14,

and 21 has been reported.

-Pneumonia.

Febrile interstitial pneumonia has been associated in infants and children with types 3 and 7.

-Keratoconjunctivitis.

-Other diseases, such as common cold, croup, bronchitis, sometimes with subsequent development of bronchiectasis, acute haemorrhagic cystitis, and encephalitis.

In healthy subjects adenovirus infection often passes without clinical symptoms. The incidence of adenovirus infection is estimated as 2-5% of the total respiratory illnesses each season. For children these figures vary from 2-24%. Two to 7% of all lower respiratory tract illnesses in young children seeking medical care can be attributed to adenoviruses.[21] [77] Adenovirus infections occur throughout the year, but incidence is higher in the cold season.

## 2-3 Influenza viruses

### 2-3-1 Introduction

Influenza, occurring epidemically, is the last great uncontrolled infectious plague of mankind. The influenza viruses, especially influenza A virus, have a special position within the group of respiratory viral agents. One of the main characteristics is the appearance of periodic epidemic outbreaks. Epidemics of influenza A and B recur with monotonous frequency. Type A and B viruses are antigenically distinct.However, within type, both surface antigens exhibit differences which confer identity and similarities which indicate lineage. Influenza C virus, isolated for the first time by Taylor in 1947[269], was found to be unrelated antigenically to type A or B viruses.

Nomenclature of influenza viruses has evolved over time. In modern terminology strain designations identify serotype -A, B, or C- , host of origin, geographic origin, strain number, year of isolation, and in parentheses numerical indices of the antigenic character of viral haemagglutinin (H) and neuraminidase (N) subtype.

2-3-2 Characteristics

The influenza viruses belong to the (ortho)myxovirus group, which are RNA containing viruses. Influenza virions are spherical particles 100 nm in diameter. The serotypes A,B, and C are determined by a soluble antigen situated as an internal antigen in the virion. In addition, there are strain-specific viral antigens in the envelope of the virion, which include haemagglutinin and neuraminidase. These proteins are responsible for the overt biological activity of each strain.

The pleomorphic influenza viruses are sensitive to inactivation by heat and aether. They are stable between pH 5.2 and pH 7.8 and more sensitive to acid than to alkaline pH.

A major characteristic of influenza A virus is its antigenic variation, presenting as minor gradual changes occurring every 2-3 years (antigenic drift) and abrupt changes at 8-12-year intervals (antigenic shift). After 30-40 years complete alterations in the surface antigens haemagglutinin and neuraminidase give rise to pandemics.[174] [175] Antigenic drift has also been recognized in type B influenza virus, but the degree of change is not great enough to permit recognition and designation of distinct subtypes.[53]

2-3-3 Laboratory methods

It is important to isolate influenza virus early in the course of an epidemic in order to identify and characterize the antigenic composition of the prevailing strain. Rhesus monkey kidney cell cultures as well as embryonated eggs are used for propagation of the influenza viruses. Rapid identification of influenza A or B virus is possible within 16 hours by immunofluorescence in cell cultures. Identification of isolates as type A or B strains is readily accomplished by the haemagglutination inhibition (HI) test. In epidemiological surveys serodiagnosis is usually employed. CF and HI tests are most commonly used, whereas neutralization and immunofluorescence are techniques not employed for routine purposes. The CF and HI tests are about equally sensitive, but use of either technique alone may miss as much as 15% of infections. Therefore, paired negative sera should be tested by the other technique. Before applying the HI test, the serum is treated with receptor

destroying enzyme, and heated at 56° C, in order to remove non-specific inhibitors.

2-3-4 Association with human illness

The clinical spectrum of influenza shows a totally asymptomatic state at one and severe illness with fatal outcome at the other extreme. Estimates of subclinical infection rates indicate that for every febrile patient there is another subject who denies illness or has slight complaints of common cold only.[109]

Uncomplicated influenza in adults appears as a febrile respiratory disease with sudden onset after an incubation period of 24-48 hours. The most frequent symptoms are fever with nonproductive coughing, nasal obstruction, headache, myalgia, malaise, chills, sore throat, conjunctivitis, and hoarseness. Anorexia, nausea, vomiting, dizziness, and insomnia may accompany fever, but diarrhoea is not characteristic. Acute symptoms last for 5-7 days. Fever usually persists for 3 days and may exhibit a diphasic course. Physical signs are few and not pathognomonic. Most patients are fully recovered within 10 days, but many experience prolonged lassitude or sense of debility and presistent cough.

The clinical picture in children tends to be milder and more difficult to recognize. Pneumonia is the most frequent complication of influenza. It should be suspected if fever persists beyond the 4th or 5th day or recurs suddenly after convalescence seems to have begun.

Viral pneumonia may account for about 20% of influenza-associated pneumonias, whereas secondary bacterial infection is more frequent[111 257] and is most commonly caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus*. Bacterial pneumonias are more frequently seen in patients with CNSLD, chronic cardiac disease, or pregnancy.

Pathologically, there is a degeneration and desquamation of the ciliated epithelium of trachea and bronchi. Among fatal cases in 1957, the dominant feature was destruction of the alveolar cell lining, with or without bacterial superinfection. From 1960 to 1970 60-70% of fatal influenza cases were caused by a superinfection due to *Staphylococcus aureus*. Data accumulated by household surveys indicate that an incidence

of 20-40% can be expected during the wave of a new pandemic. The rate of decline of intensity of epidemics is somewhat variable and irregular.

Influenza B epidemics are less frequent and it therefore seems that influenza B is less lethal than influenza A. In the last decade influenza-associated death, as judged by monthly pneumonia-influenza death rates and excess mortality rates, appears to have declined gradually. Estimations of death rates extending from 1887 through 1956 are given by Collins[48] and from 1934 through 1973 in a report by the Center for Disease Control.[34]

The possibility of prevention or reduction of severity of disease by means of vaccination is well established and beyond question.[209] However, vaccine is not always effective.[243] If the strains used for vaccination are antigenically identical with or closely related to the epidemic virus, protection can be obtained in 60-80% as compared to non-vaccinated subjects. Other more recently developed protection measures include the use of amantadine hydrochloride, but this method is not suitable for widespread application.

## 2-4 Parainfluenza viruses

### 2-4-1 Introduction

The parainfluenza viruses are species of the paramyxovirus family. In 1956 the first isolated strain was originally designated as croup-associated or CA virus[36], whereas two additional strains were characterized as HA(haemadsorption)-1 and HA-2 in 1958.[40] As these viruses had many biological properties in common, they were reclassified as parainfluenza viruses: type 1 (HA-2), type 2 (CA), and type 3 (HA-1). Type 4 was added after its first isolation in 1960.[134] During the same period, these viruses were compared with isolates obtained from animals.

The parainfluenza viruses are common causes of lower respiratory tract disease in young children and they frequently reinfect older children and adults to produce upper respiratory illnesses.

### 2-4-2 Characteristics

The pleomorphic parainfluenza viruses are RNA containing agents consisting of a nucleocapside surrounded by a lipoprotein envelope with

haemagglutinin, neuraminidase, and haemolysin activity. The diameter of parainfluenza 1, 2, and 3 viruses is approximately 110 nm, whereas type 4 measures 200 nm. The viruses are aether sensitive and acid labile. Outside the host there is a low temperature stability. Biologically, one of the main characteristics of the parainfluenza viruses is their ability to replicate in the respiratory epithelium. Excretion of virus may be prolonged up to one month or longer, even with the second or third infection[100], which can occur despite the presence of circulating antibodies.[41]

2-4-3 Laboratory methods

The 4 types of parainfluenza viruses are most readily propagated in primary monkey kidney tissue cultures, although human embryonic tissues may be used as well. Only type 2 is able to cause a characteristic cytopathogenic effect. Haemadsorption techniques are most commonly used for virus detection and identification of isolates is accomplished by inhibition of haemadsorption by specific antisera or by immunofluorescence in tissue cultures. Rapid identification can be realized by use of immunofluorescent-tagged antisera.[84] Serological tests include CF, neutralization and HI techniques. Serological methods, however, should be interpreted with care because of the occurrence of cross-reactions within members of the paramyxovirus group, which includes mumps virus as well as the parainfluenza viruses.

2-4-4 Association with human illness

Available evidence supports the view that parainfluenza viruses are ubiquitous viruses that infect most individuals during childhood. Parainfluenza viruses 1 and 2 show high isolation rates between 4 months and 5 years of age in the course of lower respiratory disease[88], whereas type 3 has been detected in all studies of hospitalized children with acute lower respiratory symptoms[20] and is recognized to be second to RS virus as a cause of bronchiolitis and pneumonia in infants.[87 88] Parainfluenza 4 infection may be common, but many of these infections are considered to be asymptomatic.

Primary infections with parainfluenza viruses are usually symptomatic

(parainfluenza 1 : 50%; parainfluenza 2 : 70%; parainfluenza 3 : 80%) but the clinical manifestations may vary strongly. The most characteristic syndrome associated with types 1 and 2 is croup or laryngotracheobronchitis. Mortality has been reported with both agents, but is rare.[69 84] Reinfection with types 1 and 2 are associated with upper respiratory symptoms not distinguishable from those due to other viruses.[90] The clinical manifestations of infections with parainfluenza 3 virus are varied. Primary infection with this type is often mild, but lower respiratory tract involvement resulting in bronchiolitis and bronchopneumonia is not uncommon.

Reinfections with parainfluenza viruses are common, but usually do not cause severe respiratory symptoms.

Efforts have been made to develop effective vaccines, especially to be used in younger children. However, all studies failed to show protection by parenterally administered vaccines despite good serum antibody responses. It would appear that attenuated strains given intranasally are more effective. Better understanding of immune response to parainfluenza virus infections is needed in order to develop effective vaccines suitable for large-scale use.

## 2-5 Respiratory syncytial virus

### 2-5-1 Introduction

Respiratory syncytial virus (RSV) was first isolated from a chimpanzee with common-cold-like illness.[193] Since its initial isolation from young children with severe lower respiratory tract disease shortly thereafter[37 42], it has become clear that RSV is the major cause of bronchiolitis and pneumonia in young infants and young children. Based on serological surveys, infection with RSV has been shown to be a common occurrence during the first few years of life.

It has been found that approximately one-half of the infants who lived through one RSV epidemic were infected, whereas infection occurred in almost all children who had lived through 2 successive epidemics.[142] Reinfection appears to be a relatively frequent event among school age children and adolescents, but not among adults.

RSV is the only respiratory agent that produces a considerable

epidemic every year in larger urban centres.[211]

2-5-2 Characteristics

RSV is an RNA containing, enveloped, pleomorphic virus with a size of 120-200 nm; it is usually classified into the subgroup of metamyxoviruses. The inner ribonucleoprotein helix has a diameter of 13 nm, intermediate between the helix of influenza viruses (9 nm) and paramyxoviruses (18 nm). RSV possesses no haemagglutinin or neuraminidase activity. The virus is infectious outside the host only if it is quickly frozen to $-70^{o}$ C. The optimum pH for storage is 7.5 and the RSV is sensitive to diethyl aether. A soluble CF antigen, different from that of paramyxoviruses and orthomyxoviruses can be separated from infected tissue culture by centrifugation.

2-5-3 Laboratory methods

RSV is labile when frozen and thawed. Bedside tissue cultivation of patient material is the most optimal way to succeed in isolation of the virus. The most sensitive cell cultures are Hep-2, monkey kidney, human amnion and human kidney in decreasing order.[299] The use of immunofluorescence to recognize viral antigens has been extensively studied.[50 280] A rise in titre of serum antibody can be shown by CF or neutralization. The CF test will detect 90% of infections among individuals older than 6 months, whereas below this age only 20% can be traced by this method. Therefore, in young infants, demonstration of infection by virus isolation is more reliable than the use of serological tests, although rises in neutralization antibodies have been detected in half of the virus-positive infections in this age group.

2-5-4 Association with human illness

The effect of RSV on the host ranges from inapparent infection to severe respiratory tract disease such as bronchiolitis or pneumonia. Illness may be gradual or abrupt after an incubation period of 3-5 days. In children the most frequent symptoms are cough, fever, rhinitis, and pharyngitis. Infection of adults often causes coryzal illness[133], but it is also an important cause of pneumonia, bronchopneumonia and bronchitis.[98]

In infants as many as 60% of cases of acute bronchiolitis have been caused by RSV. Bronchiolitis usually occurs during the first 6 months of life, when maternally derived serum antibodies are present.

Several hypotheses have been proposed to explain the severity of RSV bronchiolitis in infancy: low maternal IgG antibodies[107], low maternal $IgG_3$ subclass[89], a cell-mediated hypersensitivity reaction[237], differences in inflammatory reactions in infant lungs[228], degree of viral replication[105], and the presence of local immune complexes.[171] A high incidence of immunological disorders was reported in children with fatal lower respiratory tract infection.[2] In spite of many investigations, the pathogenesis of RSV bronchiolitis remains to be elucidated.

In the past the use of inactivated RSV vaccines has led to an exaggerated reactivity to infection[136], but nowadays encouraging attempts with attenuated, locally applicable vaccines are undertaken.

## 2-6 *Mycoplasma pneumoniae*

### 2-6-1 Introduction

Mycoplasmas are the smallest of the cellular microorganisms with known pathogenic effects in animals and humans. At the turn of the century a unique microorganism from cattle suffering from a contagious form of pleuropneumonia, was isolated. Subsequently, a number of similar agents have been found. Because of their obvious relationship to the bovine pleuropneumonia organism, they were known for many years as pleuropneumonia like organisms, PPLO. Primary atypical pneumonia, studied in some detail by Eaton and associates[65], was initially thought to be caused by a virus, known as the Eaton agent. In 1962, however, this agent was cultivated on artificial mediums and it was shown to be a Mycoplasma. The microorganism is immunologically homogeneous and has been designated *Mycoplasma pneumoniae*.

### 2-6-2 Characteristics

*Mycoplasma pneumoniae* has a diameter of about 250 nm. The cell size approaches the minimum for a free-living cell, being barely adequate to contain the genome and synthetic machinery necessary to carry out the metabolic and synthetic functions required of a minimal reproductive

unit. The pleomorphic *Mycoplasma pneumoniae* tends to be set apart from the other Mycoplasma species in that it grows relatively slowly, forming granular colonies with translucent peripheral zones, and produces haemolysis of red blood cells. The agent is resistent to penicillin, but sensitive to tetracycline and erythromycine.

2-6-3 Laboratory methods

The diagnosis of *Mycoplasma pneumoniae* infection can be made by the isolation of the agent in infusion or digest mediums enriched by the addition of serum in relatively large amounts, or by significant rises in specific antibody titre. Sputum or throat washings are inoculated in a complex medium and cultures are observed microscopically for typical Mycoplasma colonies for a period up to one month. Confirmation is achieved by demonstration of growth inhibition of isolates by specific antiserum. Furthermore, fluorescent antibody techniques are used, following inoculation into the amniotic cavity of 13-day-old embryonated eggs.

Serological diagnosis is usually achieved by CF, using a lipid antigen extracted from *Mycoplasma pneumoniae* cells. As this test reveals only 70-80% of infections, more complex techniques like immunofluorescence can be added in order to increase serological test sensitivity.

2-6-4 Association with human illness

*Mycoplasma pneumoniae* can be associated with pharyngitis, tracheobronchitis, pneumonia or a combination of these syndromes. Respiratory symptoms are often preceeded by general complaints such as malaise, fatigueness, headache and moderate fever. In case of pharyngitis, sore throat, coughing and slight rhinitis are common complaints and diffuse erythema of pharynx and tonsils is usually found. Tracheobronchitis is associated with persistent coughing, most often in combination with substernal pain, and thorax X-rays frequently reveal pulmonary shadows.

With the appearance of respiratory symptoms, malaise and headache increase in severity and non-productive coughing in combination with fever and myalgia is a prominent feature. In relation to sometimes extensive radiographic changes, the clinical symptoms are scarce. There is a great

variety of radiologic patterns from interstitial, disseminated infiltrates to total lobar consolidation[38] and radiologic changes can be found for a period of several weeks.[23]

Reconvalescence is often prolonged, although fever usually subsides within 3-10 days. *Mycoplasma pneumoniae* bears a low mortality. Other syndromes that have been related to *Mycoplasma pneumoniae* comprise meningoencephalitis, myocarditis, pericarditis, Guillain-Barré syndrome, and Stevens-Johnson syndrome.

## 2-7 *Chlamydia psittaci*

### 2-7-1 Introduction

Chlamydiae are non-viral microorganisms containing DNA as well as RNA and resembling rickettsiae in many respects. The genus is composed by 2 groups: *Chlamydia trachomatis* and *Chlamydia psittaci*. Psittacosis occurs in psittacine birds and the closely related, if not substantially identical ornithosis occurs in wild birds as well as domestic fowl, such as pigeons and canaries. Human infection is most commonly acquired by such reservoirs of infection; transmission between humans has been described, but is very rare.

### 2-7-2 Characteristics

Chlamydiae are characterized by an intracellular developmental cycle, which is not completely understood yet.[56] The mature particle, or elementary body is spherical or coccoidal in shape and relatively large, 200-500 nm. Elementary bodies are capable of infecting new cells. The chlamydiae possess 2 types of antigens, group and specific, both present in the cell wall. The group antigen is heat-stable and glycolipid in nature and induces CF antibodies. The specific antigens are relatively labile and soluble in alkali; they are probably proteins. Specific antigens are demonstrable by fluorescent antibody methods and strains are usually typed in this way.

### 2-7-3 Laboratory methods

Isolation of the infectious agent is very difficult in case of human infection, whereas material from birds more readily yields growth of

the organism. The agent is best grown in yolk sac of embryonated eggs. Serodiagnosis is used in practice and is dependent on the CF reaction, carried out with paired sera to demonstrate rise in titre.[56] [97]

2-7-4 Association with human illness

Human illness is produced by inhalation of infected material. The disease in man is a pneumonia with an incubation period of 1-2 weeks. The onset may be sudden or insidious. The symptoms include chills and fever, photophobia, usually severe headache, anorexia, sore throat, nausea,and vomiting. A dry cough develops, which persists and may become more severe; cyanosis and low blood pressure are frequent, and disorientation and occasional delirium indicate involvement of the central nervous system. Myocarditis can occur, occasionally with fatal outcome. X-ray examination of the thorax shows migrating patchy areas of consolidation in one or both lungs. Leucocytosis does not occur until late in the disease or early convalescence. *Chlamydia psittaci* usually causes a more severe clinical picture than *Chlamydia ornithosis*. Reinfections can occur. Tetracyclines are most frequently used for therapy of infections by chlamydiae.

# CHAPTER 3
# THE ROLE OF RESPIRATORY INFECTION IN THE PATHOGENESIS OF CNSLD

## 3-1 Introduction

The role of respiratory infection in the pathogenesis of CNSLD has been discussed extensively in the past decades. Much work in this field, especially with regard to viral-bacterial interrelationships in the pathogenesis of "chronic bronchitis" has been done by Stuart Harris and coworkers.[255 256 258] Nowadays it is generally believed that there is no support for a role of respiratory infections experienced in adult life in the progressive obstructive airway disease found in some patients.[73 119 153 266] On the contrary, there is growing interest in the field of paediatric respiratory illness in relation to CNSLD. Over the past few years a number of epidemiological and pathophysiological studies have appeared, suggesting a close relationship between lower respiratory tract illnesses in infancy and early childhood and chronic respiratory disorders and pulmonary function abnormalities in later life. The backgrounds of this relationship are not fully understood yet. The association could reflect some inherent genetically determined defect of the airways, rendering them susceptible to infection and other adverse effects at all ages. It is also possible that subtle changes, induced by childhood infection in the growing airways, predispose them to risk effects by leading to bronchial hyperreactivity or by reducing the effectiveness of pulmonary defense mechanisms. Some studies indicate that both endogenous and exogenous factors are important, respiratory infection being a trigger mechanism for the appearance of symptoms in genetically predisposed persons.[71 81]

In this chapter a short review on the role of infection in the pathogenesis of CNSLD will be given with special emphasis on the importance of paediatric illnesses. This is difficult because the exact nature of the childhood illness is often unknown. Moreover, semantic uncertainty with regard to the term respiratory infection[96 206] and the different

diagnoses within the spectrum of CNSLD[233] (chapter 1) makes it arduous to ascertain the above-mentioned relationship. As many studies are limited to asthma or chronic bronchitis-emphysema, the literature will be discussed for these categories separately, although this procedure brings some overlap of data along with it.

## 3-2 Respiratory infection and asthma

### 3-2-1 Terminology

The term infectious asthma, still to be found in reference books on bronchial asthma[233 263], underlines the role infection is supposed to play in the pathogenesis of the disease. This designation has been used widely, if respiratory infection was thought to be the main provoking factor of asthmatic attacks, occurring in about 20% of the patients according to old studies.[213 225] Nowadays, the term has been abolished by many authors because infections can cause exacerbations in patients with extrinsic as well as intrinsic asthma and other provoking factors are demonstrable in most instances.[236 276]

Similarly, the diagnosis "bacterial asthma", which has been used synonymously, is not commonly used any more, as substantial evidence for bacterial allergy in bronchial asthma is absent.[66 140 304] The beneficial effects of bacterial vaccines in the treatment of asthma, reported by some authors[51 52 102 262] is probably due to the production of interferon, according to Singer *et al.*[239] In children a distinction between wheezy bronchitis (bronchial obstruction in the course of respiratory infection) and asthma (other factors provoking obstruction as well) is sometimes made. Although slight differences are demonstrable between these clinical entities[116] the pathophysiological substrate is supposed to be identical.[294]

### 3-2-2 Childhood respiratory infection

#### 3-2-2-1 Subglottic laryngotracheobronchitis (croup)

Croup has been considered a disease process of the larynx, trachea, and, possibly, larger bronchi. Therefore, it has never been regarded as a forerunner of bronchial obstructive disease. However, physiological changes indicating small airways involvement, were observed in the

course of the disease by Newth *et al.*[199] and Taussig and co-workers.[268] Subsequently, a high incidence of increased bronchial reactivity to exercise[163] and metacholine[103] was noticed in children with history of laryngotracheobronchitis.

Hyperreactivity occurred irrespective of a positive history of allergy and a family background with regard to atopic disease. Although symptomatic asthma was not notified in the children studied, these findings suggest that subjects with a history of croup constitute a population susceptible to risk factors associated with bronchial obstructive disease.

3-2-2-2 Bronchiolitis

Acute bronchiolitis is a syndrome of inflammatory obstruction at the level of small airways, characterized pathologically by necrosis of bronchiolar epithelium followed by lymphocytic infiltration of the bronchial wall and narrowing of the lumen.[1] The majority of patients are children in the first two years of life and respiratory syncytial virus has been shown to be the most frequent aetiological agent.[11 39 142] Freeman *et al.*[80] in a retrospective study found that 50% of children with lower respiratory tract infections continued to have wheezing episodes or allergic manifestations, whereas a percentage of only 17 was noted in children with upper respiratory tract infections.

Several studies have confirmed that there is a high incidence of recurrent wheezing subsequent to acute bronchiolitis. In Table 3-1 a summary of these studies is given. Incidences of recurrent wheezing after bronchiolitis vary from 32 to 57%. Zweiman *et al.*[306] found this percentage to be constant when their patients were reexamined some years later.

It has been noticed that the association of bronchiolitis with asthma is, among other factors, related to a positive family history of allergy and the suggestion has been made that the episode of bronchiolitis is in fact the infant's first asthmatic attack in many cases.[169 232] A hypothesis that only children with bronchiolitis not caused by RSV are predisposed to subsequent asthma was put forward by Simon and Jordan.[238] This hypothesis was corroborated by a Swedish report of elevated IgE

Table 3-1 Results of follow-up studies, indicating different incidences of recurrent wheezing subsequent to acute bronchiolitis

| Authors | No. of patients | Follow-up period (yrs.) | % with allergic family history | % with recurrent wheezing |
|---|---|---|---|---|
| Eisen *et al.*[67] | 63 | 1- 7 | 44 | 32 |
| Hyde *et al.*[122] | 77 | ½- 2 | 70 | 40 |
| Wittig *et al.*[295] | 100 | 4-14 | 29 | 46 |
| Zweiman *et al.*[305] | 24 | 3- 4 | 50 | 50 |
| Rooney *et al.*[232] | 62 | 2- 7 | 54 | 57 |

levels in sporadic and normal levels in epidemic bronchiolitis[220], but viral investigations were insufficient in this study.

Some recent, well controlled studies have added substantial information. Kattan *et al.*[138] have shown that in subjects without respiratory symptoms studied 10 years after an episode of bronchiolitis a high incidence of minor pulmonary function abnormalities are observed, indicating the presence of a residual parenchymal or airway lesion.

This observation was confirmed in follow-up studies by Pullan *et al.*[222] and Mok *et al.*[189] In both studies a significantly higher number of children with a history of bronchiolitis had alterations in lung function as compared to matched control subjects. Furthermore, a high incidence of airway hyperreactivity to histamine and exercise was observed following bronchiolitis. However, no excess of allergic manifestations or family atopic background was found and no differences were found between RSV positive and RSV negative bronchiolitis patients.

### 3-3 Respiratory infection and chronic bronchitis

#### 3-3-1 Epidemiological studies

There are numerous factors which are related to the development of chronic obstructive lung disease. Beside genetic determinants, these include area of residence, air pollution index, family's social class, family size, smoking habits, and the history of lower respiratory tract illness in early childhood. However, when all these factors are taken

into account, the aetiological picture remains incomplete.

The importance of respiratory tract infection in childhood in the pathogenesis of adult CNSLD has been stressed in many epidemiological studies. Serious respiratory tract infections, including bronchiolitis in childhood, were supposed to have occurred in a high proportion of adult New Guinea primitive tribal people with chronic cough and diminished lung function.[296] Colley and co-workers[47] in a prospective cohort study examined the prevalence of cough in young adults whose respiratory status in early life had been documented. More cough was noted in subjects with lower respiratory tract illness in infancy, even after accounting for smoking habits. This study confirmed older follow-up studies by McDonald *et al.*[168], Reid and Fairbairn[227], and Case *et al.*[31 32] Respiratory illness early in life is supposed to be associated with an increased susceptibility to recurrence of respiratory symptoms in later childhood.[17] Increased respiratory infection rates in patients with CNSLD have been found by several investigators.[165 182 190 291] Monto and Ross[191] suggest on the base of their findings that acute infection may play an important role in the pathogenesis of chronic respiratory disease.

3-3-2 Physiological studies

There are several surveys in which physiological parameters have been used to assess airway function in subjects with a history of childhood respiratory illness. Holland *et al.*[112] found that in a group of children aged 5-14 years, peak flow rates were adversely affected by a past history of pneumonia or bronchitis. A significant correlation between abnormalities in forced expiratory volume (FEV) and a history of frequent cough, colds going to the chest, and episodes of lower respiratory tract illness was reported by Lunn *et al.*[167] Indications for residual damage to airways subsequent to lower respiratory tract illnesses were also found by Leeder an co-workers, using flow-volume curves and closing volume tests.[154]

Recent data confirm the suspected relationship between paediatric respiratory disorders and the subsequent development of airway disease in adults. Burrows *et al.*[26] in a retrospective study showed that

subjects with a history of paediatric respiratory illness had only relatively mild impairment of ventilatory function at young adult age. However, these persons showed excessive decline in lung function with advancing years and with cigarette use, factors known to be of the utmost importance in the development of progressive airflow obstruction.[73]

It is possible that these data similarly apply to the asymptomatic subjects with minor lung function deteriorations and increased bronchial reactivity to various stimuli, subsequent to croup (chapter 3-2-2-1) and bronchiolitis (chapter 3-2-2-2), in whom the infectious origin of paediatric illness was established.

# CHAPTER 4

# VIRAL RESPIRATORY INFECTIONS AND EXACERBATIONS OF CNSLD

## 4-1 Introduction

In the late fifties, when Asian influenza was epidemic, several reports on the possible asthmatogenicity of the influenza H2N2 virus appeared.[164 219 224 226 287] Numerous studies have been undertaken since which show that various respiratory viruses as well as *Mycoplasma pneumoniae* are able to cause exacerbations in patients with CNSLD.

The majority of patients with CNSLD tend to react with an exacerbation in the course of a respiratory viral infection. Table 4-1 summarizes the results of the studies which support this view.

Table 4-1 Frequency of exacerbations in CNSLD during respiratory viral infections. Results of various studies

| Authors | No. of viral infections | Percentage with exacerbation |
|---|---|---|
| Stenhouse[251] | 16 | 50 |
| Minor *et al.*[183] | 32 | 53 |
| Stark *et al.*[249] | 23 | 57 |
| Stenhouse[250] | 13 | 62 |
| Minor *et al.*[184] | 38 | 63 |
| Löwenberg and Orie[166] | 14 | 64 |
| Huhti *et al.*[121] | 43 | 77 |
| McNamara *et al.*[172] | 34 | 79 |
| Eadie *et al.*[64] | 17 | 82 |
| Total (studies combined) | 230 | 67 |

In most studies the population of patients is limited to the asthma -or the chronic bronchitis- group. In both groups the significance of bacterial infections in causing exacerbations seems to be of little

importance.

Several investigations in asthmatics suggest that there is no relationship between bacterial infection of the respiratory tract and the occurrence of exacerbations.[13 70 148 170 184 235] This applies also to the patients defined as chronic bronchitics.[25 54 101] In view of these data and the emphasis of this thesis on respiratory viruses, the subject of bacterial infection will not be dealt with to further extent, whereas the literature on viral agents and *Mycoplasma pneumoniae* will be discussed for asthma and chronic bronchitis apart.

4-2 Viral respiratory infections in the course of bronchial asthma

Berkovich *et al.*[12] were the first to report a prospective study on viral and *Mycoplasma pneumoniae* infection causing exacerbations in asthmatic children. An attempt was made to culture viruses from the throat during attacks of asthma, but sampling was infrequent and few agents were isolated. However, it was concluded from serological tests that 33 out of 108 exacerbations occurred in the course of a viral infection. Influenza A, parainfluenza 3 and *Mycoplasma pneumoniae* accounted for the majority of these episodes.

In an earlier period asthmatogenetic properties have been assigned to RSV, parainfluenza 1 and 3 viruses, and adenovirus by Freeman and Todd[80] and to *Mycoplasma pneumoniae* by Hers and Mulder[110] on the base of retrospective data.

In the seventies several authors reported on the subject with divergent conclusions. The majority of studies are dealing with asthmatic children, whereas data in adults are scarce. In children Disney *et al.*[57] found evidence of viral infection in a minority of patients only. In 5 of 51 children with an acute attack of asthma, a viral agent could be identified. However, the study is incomplete, because serological tests were done in 26 of the children only.

A somewhat higher percentage was reported by Mitchell *et al.*[185] who found an infection rate of 17% in 267 children admitted to hospital because of wheezy bronchitis or asthma. Virus isolation was significantly more common in readmissions than in first admissions. Lambert and Stern[148] described a seven-year-old asthmatic boy in whom 3 virus iso-

lations were made in the course of 5 asthmatic episodes.

In a prospective study of viral respiratory infection in 32 one- to five-year-old children, hospitalized in the National Jewish Hospital in Denver, McIntosh *et al.*[170] found 139 episodes of wheezing, 58 of which (42%) were associated with identifiable viral infection. Of 25 infections by RSV, 24 caused exacerbations, whereas influenza A-H3N2 virus was not associated with wheezing in any of 11 children.

Minor *et al.*[184] followed 16 children, aged 3 to 11 years, with non-allergic asthma, who experienced 61 episodes of wheezing. Forty-two of these were coincident with an apparent symptomatic respiratory infection (SRI). Evidence of viral infection was found in 24 patients, rhinovirus being the agent most frequently isolated. All of 6 influenza A-H3N2 episodes were associated with attacks of asthma. Asthma was precipitated during 21 of 23 severe SRIs of viral origin, whereas no asthma was noted in episodes of asymptomatic virus shedding. The same authors stress the importance of rhinovirus and influenza A-H3N2 in another study[183], in which adult patients also participated. Seventeen out of 24 (71%) episodes of SRI by rhinoviruses and 4 of 5 influenza A infections were accompanied by wheezing. A high incidence of wheezy episodes during rhinovirus infections in asthmatic children was also reported by Gregg[95] who found that 46 out of 57 (80%) of these infections were associated with acute wheezy bronchitis.

In a study of 51 asthmatic adults, Clarke[45] suggested that respiratory infection is responsible for a small part of asthmatic flare-ups only. During 111 exacerbations proof of viral infection was found in 8 of these only. However, like in the study by Disney *et al.*[57], serological investigation seemed to be incomplete.

Higher figures were given in a Finnish study[121] in which 27 of 142 patients who were admitted to hospital because of severe asthmatic attacks showed evidence of viral and *Mycoplasma pneumoniae* infection. There were no differences between allergic and non-allergic patients and the use of oral corticosteroids did not influence the frequency of infection. Comparable data were given by Miguères *et al.*[180] who found seroconversion in 54 out of 211 exacerbations in 178 adult patients. Influenza A and *Mycoplasma pneumoniae* were the agents most frequently

involved.

Finally, Lambert and Stern[148] reported on a 25-year-old female in whom 2 virus isolations were made in the course of 6 asthmatic episodes.

The results of the above mentioned studies are summarized in table 4-2.

Table 4-2 Summary of studies on the relationship between respiratory viral infection and excerbations in asthmatics

| Authors | No.of patients | Age | No.of exacerbations | % with viral infection | Main agents |
|---|---|---|---|---|---|
| Clarke[45] | 51 | average:35 | 111 | 7 | |
| Disney *et al.*[57] | 51 | ?(children) | 51 | 10 | |
| Mitchell *et al.*[185] | 192 | children>1 | 360 | 17 | RSV,rhino |
| Huhti *et al.*[121] | 63 | 15-77 | 142 | 19 | Infl.A, RSV *M.pneum.* |
| Miguères *et al.*[180] | 178 | 15-75 | 211 | 26 | Infl.A, *M.pneum.* |
| Berkovich *et al.*[12] | 84 | 0-16 | 108 | 31 | Infl.A, para-infl., *M.pneum.* |
| Minor *et al.*[184] | 16 | 3-11 | 61 | 39 | Rhino, infl.A |
| McIntosh *et al.*[170] | 32 | 1- 5 | 139 | 42 | RSV, para-infl.2, corona |
| Lambert & Stern[148] | 2 | 7-25 | 11 | 45 | Rhino |

4-3 Viral respiratory infections in the course of chronic bronchitis

It has been known for a long time that flare-ups of chronic bronchitis can occur in combination with rises in antibody titre to influenza A[259] and adenovirus.[279] The first reported attempts to isolate a viral agent during exacerbations in chronic bronchitics were by Jack and Gandevia[125] and Hennessy[108], both of whom failed to identify any viruses. However, Carilli *et al.*[30], who found serological evidence of viral infection in 24 out of 46 exacerbations, succeeded to isolate a viral agent in 4 cases. RSV was the most common agent in this study, followed by influenza and the non-viral agent *Mycoplasma pneumoniae*. The importance of the RSV was also stressed in a study by Sommerville[244], who reported that in 41 out of 82

seroconversions to this agent the clinical diagnosis of an acute bronchitic exacerbation was assessed.

Stark *et al.*[249] reported that serological tests revealed 13 influenza B infections associated with 9 exacerbations and 10 parainfluenza infections with 4 flare-ups in 199 patients suffering from chronic bronchitis. These viruses accounted for only 7% of exacerbations experienced by these patients.

Similar data were provided by Moffat and Sutherland[188] who calculated that only 3 of a total of 68 exacerbations were related to viral infections. In this study the sera from 20 male bronchitics were investigated at 8-week intervals for a period of more than 4 years. Only 6 significant seroconversions were found which could be due to the relatively long intervals.

Higher figures were given by Eadie *et al.*[54], who followed 15 patients with chronic bronchitis every fortnight for a period of almost $2\frac{1}{2}$ years. They identified 16 virus infections in the course of 75 exacerbations, rhinoviruses being the most frequently isolated agents.

Stenhouse[250] also succeeded in isolating rhinoviruses in 8 of 34 patients. The infections accounted for 14% of the exacerbations during the study period. In another 64 flare-ups experienced by these patients, 7 seroconversions to a viral agent and 1 to *Chlamydia psittaci* were found.[251]

Similar figures were presented by Fisher *et al.*[72], who identified 7 viral and 2 *Mycoplasma pneumoniae* infections in the course of 63 exacerbations in 23 male bronchitics and by Ross *et al.*[234], who found fourfold or greater rises in antibody titre in 20 out of 125 exacerbations, using serological methods only.

Lambert and Stern[148], in a study mentioned before, found evidence of viral infection in 8 out of 30 deteriorations of chronic bronchitis in 6 patients. Mufson *et al.*[194] imputed one-fifth of the 153 exacerbations studied by them to viral or *Mycoplasma pneumoniae* infections, whereas Gump *et al.*[101] were able to relate one-third of 116 periods of increased cough and sputum production in 25 patients to respiratory viruses and *Mycoplasma pneumoniae*.

There are two reports in which a majority of bronchitic episodes could

be associated with viral or *Mycoplasma pneumoniae* infection. McNamara *et al.*[172] found an association with rhinovirus (18 times), RSV (5 times) and *Mycoplasma pneumoniae* (4 times) in 27 out of 42 episodes of increased bronchitic symptoms. Of 34 infections by these agents, 79% were related to excerbations.

In a Belgian study Lamy *et al.*[149] reported 49 exacerbations experienced by 44 of 111 patients they observed for periods ranging from 1 to 9 months. A diagnosis of viral infection was made in 31 cases (63%) by isolation and/or seroconversion, including 1 infection by *Coxiella burneti* and one by *Mycoplasma pneumoniae*. There was a remarkable relationship between parainfluenza infection and periods of increased symptoms of chronic bronchitis. The results of the above mentioned studies are summarized in table 4-3.

Table 4-3 Summary of studies on the relationship between respiratory viral infections and exacerbations in chronic bronchitics

| Authors | No.of patients | Age | No.of exacerbations | % with viral infection | Main agents |
|---|---|---|---|---|---|
| Moffat *et al.*[188] | 20 | 40-59 | 68 | 4 | |
| Stark *et al.*[249] | 199 | 20-79 | 185 | 7 | Infl.B, parainfl.3 |
| Stenhouse[251] | 34 | 51-73 | 64 | 12 | Infl.A,B |
| Stenhouse[250] | 34 | 51-73 | 56 | 14 | Rhino |
| Fisher *et al.*[72] | 23 | 44-67 | 63 | 14 | Infl.A, *M.pneum.* |
| Ross *et al.*[234] | 172 | > 40 | 125 | 16 | Infl.A,C,RSV |
| Mufson *et al.*[194] | 45 | ? | 153 | 20 | Infl.A, parainfl. |
| Lambert & Stern[148] | 6 | 46-74 | 30 | 27 | Parainfl.1,3 |
| Eadie *et al.*[64] | 15 | 25-84 | 47 | 28 | Rhino |
| Gump *et al.*[101] | 25 | 31-72 | 116 | 34 | Infl.A, parainfl.3,RSV |
| Carilli *et al.*[30] | 30 | 26-80 | 46 | 52 | RSV, Infl.A, *M.pneum.* |
| Lamy *et al.*[149] | 111 | 30-80 | 49 | 63 | Infl.A,B, parainfl. |
| McNamara *et al.*[172] | 29 | 19-75 | 42 | 64 | Rhino, RSV, *M.pneum.* |

# CHAPTER 5
# CNSLD AND HIGH MOUNTAINS

## 5-1 Introduction

It is a widely known clinical phenomenon that patients with CNSLD, especially those with distinct asthmatic symptoms, react favourably upon moving to the high mountains. The first reports, cited by Van Geuns[86], date from Denz and Spengler, both of whom described in 1878 some asthmatic patients with a remarkable improvement of their bronchial obstructive disease during their stay in a high mountain climate. Numerous reports on the subject have been published since and the literature until 1956 has been reviewed in detail by Van Geuns.[86]

In this chapter some hypotheses regarding the beneficial effects of climatic factors on CNSLD will be discussed briefly, completed by some of our own clinical observations.

## 5-2 Theoretical considerations

The various theories on the favourable effect of the high mountain climate in CNSLD patients can be roughly divided into 3 groups.

a. The first group emphasizes the change of respiratory mechanisms in the thinner air, containing less oxygen. Especially Jaeger[127 128] pointed out that there is a correlation between the density of inhaled gas and the resistance in the airways. Inspired air at an altitude of 1500 m has a density of 83% as compared to 100% at sea-level. This relatively low density could be partly responsible for the beneficial effects of the high mountain climate. The use of light gas mixtures, such as helium-oxygen (80%-20%) in lung function studies and the therapy of asthma[10] is based on the same physical principles.

b. The second group stresses the importance of biometereological influences. Mörikofer[192] gave a detailed review on the metereological circumstances of Davos, emphasizing the low wind intensity, the

radiation conditions, especially with regard to ultraviolet light, the relatively scanty rainfall and low humidity, as well as the low oxygen content of the inspired air. Tromp and Bouma[275] suggested that there is an association between sudden increases in atmospheric turbulence and asthmatic attacks and supposed that the susceptibility of asthmatic patients to these atmospheric changes is attributable to a dysfunction of hypothalamic thermoregulatory nuclei in these subjects.

The stability of atmospheric conditions and the relative absence of sudden rises in turbulence could possibly account for the low frequency of asthmatic attacks observed in Davos.[302]

c. The third and probably most important group emphasizes the absence or at least much smaller frequency of bronchial irritants, such as inhalation allergens. Over half a century ago Storm van Leeuwen[253 254] reported that asthmatics with a distinct allergy to house-dust were free of complaints when staying in an alpine climate. In 1924 this investigator took with him on a journey through Switzerland 3 patients suffering from asthma and studied the condition of these persons in Basel, Lugano, Ragaz, Vulpera, Davos, and St. Moritz, respectively. At Davos one of these patients, who was without complaints there, was subjected to a test in which by means of inhalation of dust from Holland an asthmatic attack was provoked. Varekamp[281] tested the allergenic effects of the house-dust collected during this trip on Dutch asthmatic patients and found strong, negative correlation between the allergenic potency and the altitude at which the house-dust was collected. (Table 5-1).

Van Geuns[85] submitted 35 patients to inhalation tests with equally prepared Dutch and Davos house-dust extract. Eighteen of these patients showed a positive reaction to Dutch house-dust, whereas only one of them had a positive reaction to the Davos extract. Voorhorst and Spieksma corroborated these findings by some elegant studies in the sixties.[246 247 282 283] It was found that a mite, *Dermatophagoides pteronyssinus*, is of particular importance with regard to the allergenic potency of house-dust and that its ideal growth conditions are high humidity and a temperature of approximately 25° C. This probably explains why house-

Table 5-1 Percentage of patients with positive skin tests to extracts of house-dust, collected at different altitudes

| House-dust from | Altitude (m) | % of Dutch patients with positive skin test |
|---|---|---|
| Holland | 0 | 83 |
| Basel | 100 | 67 |
| Lugano | 300 | 30 |
| Ragaz | 800 | 35 |
| Vulpera | 1200 | 18 |
| Davos | 1500 | 12 |
| St.Moritz | 1800 | 7 |

According to Varekamp[281]

dust allergy is often troublesome with old bedding, in damp houses, and during the wetter months of the autumn and winter.[176]

The low humidity in alpine climates, e.g. at Davos[192], probably accounts for the much lower levels of contamination in the Swiss mountains.[248] Other approaches of comparing allergenic potencies of house-dust from alpine and lowland regions, e.g. chemical investigations by Berrens *et al.*[14] have yielded similar data. Davos house-dust turned out to have much lower allergenic activity than Dutch samples.

In addition to the relative absence of the house-dust mite, other inhalation allergens, like moulds and pollens have been shown to occur less frequently in high mountain climates.[159 298]

5-3 Comment

The numerous reports on the favourable influence of high mountain climate on CNSLD are confirmed by our own observations. Especially the data described in chapter 5-2 c. are in agreement with our experiences with house-dust allergic patients. We found a significantly greater frequency of improvements in asthmatics with a distinct house-dust allergy as compared to matched non-allergic patients (Table 5-2). Moreover, most spectacular results have been obtained in young children in whom the factor allergy frequently is very pronounced. Severe asthmatic

Table 5-2 Results of house-dust allergic asthmatics as compared to non-allergic patients in Davos (according to Roldaan and van Rijn[231])

| | No. | No. with definite improvement in $FEV_1$ | Percentage |
|---|---|---|---|
| Patients allergic to house-dust | 21 | 19 | 90 |
| Patients non-allergic to house-dust | 20 | 6 | 30 |

children, requiring oral corticosteroids in Holland, have been shown to manage without those medicaments within a short time when staying in the alpine climate of Davos.[141]

Furthermore, other patients suffering from obstructive disorders of a more irreversible character also tend to have fewer complaints. In a study by Arblaster and Zuidema[7] 83% of asthmatic patients showed an improvement, whereas 44% of chronic bronchitics did.

It has been frequently noticed that subjects with marked hypersecretion produce much less sputum during their stay in Davos. This might be associated with the statement of many patients that they experience fewer respiratory infections while staying there. Although some authors, like von Neergaard (cited by van Geuns[86]), drew attention to this infrequency of infective episodes long ago, there is no conclusive evidence in literature that respiratory infections occur less often in alpine climates. Similarly, literature does not provide reliable data on the course of bronchial hyperreactivity in patients with CNSLD staying in alpine climates. Investigations into this field should be subject of future research activities.

# CHAPTER 6
# DAVOS, THE NETHERLANDS ASTHMA CENTRE AND ITS PATIENTS

## 6-1 Davos and the Netherlands Asthma Centre

Davos, a village with approximately 12,000 inhabitants, is situated at an altitude of 1560 m in "Graubünden", which is the largest of the 22 Swiss cantons and is located at the eastern border of the country. Davos is surrounded by mountains, which makes it sheltered from wind to a great extent. Its favourable weather conditions and the extensive facilities, especially in winter, have turned the place into one of the most prominent wintersport areas of Europe. Moreover, because of its metereological advantages as compared to the conditions in the lowlands (see chapter 5), the village has achieved a reputation as a leading "Kurort". At the end of the past century the relatively favourable effect of the alpine climate in the treatment of pulmonary tuberculosis arose interest in Holland and private initiatives led to the foundation of the Netherlands Sanatorium, which was opened in 1897. For a period of over 70 years Dutch tuberculosis patients were treated in the Sanatorium, but with the development of tuberculostatic medicaments and because of better hygienics the disease gradually got more or less overcome.

Because of the good results, described by others, in patients suffering from asthma and bronchitis, a start was made with the treatment of these patients in the late fifties. In the course of time CNSLD patients have replaced tuberculosis patients and, accordingly, the clinic's name was changed into Netherlands Asthma Centre.

## 6-2 Patients

### 6-2-1 General considerations

Within the Dutch medical care for patients suffering from CNSLD the clinic in Davos serves more or less as a terminal station, suitable for patients in whom good control of symptoms of the bronchial obstructive

disorder cannot be accomplished despite intensive medical treatment in The Netherlands.

Severe bronchial asthma is the diagnosis most commonly applied to the subjects referred to the centre, but other conditions within the spectrum of CNSLD as well as bronchiectasis of known and unknown origin are also frequently seen. Because almost all of the patients have a long history of severe disease when they are sent to Davos, serious interference in daily life before referral is frequently seen and considerable psychosocial implications of the illness are rule rather than exception. Therefore, an integral, holistic approach of treatment was chosen as a model some years ago, as described by Lansen,[150] with a team comprising specialists from different therapeutic fields for each group of patients. Within this model, starting from a usually considerable somatic amelioration, thanks to extensive medical care and climatic factors, patients are enabled to explore possibilities of improving their way of life in different respects.

Subjects are divided into groups, mainly according to age. These groups have their own living units, which is of epidemiological importance in view of the survey described in this thesis. Patients participating in the study were, to a major extent, recruited from three groups, which will be discussed briefly.

6-2-2 Group A - the "Senioren"

This group comprises 20-25 patients of about 35 years of age onward, suffering from different conditions covered by the term CNSLD. On the one hand there are subjects to whom the term asthma can be applied, with complete reversibility of their bronchial obstructive disorder, on the other hand there are patients who are seriously disabled because of severe irreversible obstructive changes. The majority of persons can be regarded as asthmatic, a substantial part of them having no allergic features (intrinsic asthma).

The therapeutic aims for this group can be roughly described as physical and mental rehabilitation. The time usually necessary to achieve these aims varies between 3 and 6 months, but longer periods are not infrequent.

Apart from medical care, the daily therapeutic programme for these patients consists of physical and movement therapy, creative therapy, group sessions twice weekly, house-keeping activities and, if necessary, individual psychotherapeutic support. Within movement therapy much attention is given to physical fitness and relaxation techniques, including autogenic training.

Within this group, readmissions are not infrequent and some of the elderly patients with chronic airflow obstruction are referred to the clinic every year in winter for a short rehabilitation. Since 1977 these patients form a special group, usually called "overwinteraars" or "veteranen".

6-2-3 Group B - the "Junioren"

This group consists of about 15 adolescents and young adults, mostly between 17 and 30 years of age. The diagnosis extrinsic atopic bronchial asthma can be applied to most of them; in some of these subjects severe irreversible features are already present. It is almost characteristic that these patients show signs of retardation in different ways, due to severe chronic illness. This goes especially for self-reliance and acceptance of responsibilities. Therapy is directed toward physical rehabilitation and mental growth, aiming to render these patients independent, self-supporting people, who can manage to live with a chronic disease satisfactorily. It has become clear that group-directed activities can be used as a therapeutic tool in this respect.

The programme offered to these patients does not differ essentially from that of group A, but is more intensive and has more educational aspects. The average duration of treatment varies between 6 and 12 months.

6-2-4 Group C - the "Vossen"

This group comprises 10 children of 9 to 14 years of age, almost all of them suffering from severe extrinsic atopic bronchial asthma with (almost) complete reversibility of lung function. Asthmatic symptoms in most of these children used to persist, despite intensive medical care, in Holland and many of them were residents of Dutch asthma hospitals for

several years. These patients are frequently referred to the high mountain climate because of the impossibility to avoid the use of oral corticosteroids in relatively high dosages for longer periods of time. A dramatic spontaneous improvement is usually seen and most of the children manage to be completely or almost totally symptom-free without the use of oral steroids. The house they are living in is somewhat remote from other living units in order to create a domestic atmosphere, a home of their own.

Life is as normal as possible, school being the most time-consuming element of the day for these children. Within the daily programme, there is much attention for sports and fitness training, because of the sometimes severe motoric retardation. There are 4 pedagogically trained leaders, 2 females and 2 males, for this group. Parents are involved in therapy as much as possible.

In order to obtain a better prognosis for these patients, who were severely disabled in Holland, it is necessary for them to stay for a relatively long period. After a stay of about 2 years improvement of their condition is maintained in approximately 70% of the children.[3] In November 1978, a new group -the "Tussengroep"- was instituted, formed by 8 children, aged 14-17 years. Some of these patients participated at the end of the study and were incorporated with the subjects of group C.

# PART II

# CHAPTER 7
# PATIENTS, MATERIAL AND METHODS

## 7-1 Introduction

The study of viral respiratory infections in patients of the Netherlands Asthma Centre was conducted for a period of 3 years and 7 months, from September 15, 1975 to April 15, 1979. The aim of the investigation was explained to the subjects and consent to participation was given by all patients without restraint. Principally, every patient with CNSLD referred to the clinic could enter the study, but a total number of 40 to 45 participants at any given time was aimed at. Because of the much greater turn-over of the group A patients, most participants came from this group, whereas the number of children in group C was relatively small. On the other hand, the follow-up periods of the latter patients were much longer.

## 7-2 Patients

### 7-2-1 Classification of patients

In order to investigate whether various patterns of reaction to respiratory infection can be found in patients with different types of CNSLD, the patient population was divided into 3 groups, maintaining the classification in groups A, B, and C as described in chapter 6-2. Patients were classified mainly on the base of anamnestic data and reversibility of lung function, but other parameters like physical examination, skin tests, total peripheral eosinophilic leucocyte counts, chest röntgenograms, and histamine threshold were also taken into account. Special attention was given to the occurrence of obvious asthmatic attacks with wheezing, alternated with symptom-free intervals either at present or in the past. Patients were asked to tell whether their main complaint nowadays was paroxysmal dyspnoea or progressive breathlessness on exertion and the importance of coughing and phlegm production as a symptom was judged.

Lung function measurements, including static lung volumina, $FEV_1$, flow at various lung volumes, and airway resistance were done at least once in 4 weeks in all patients before and after bronchodilation using a bodyplethysmograph (Bodytest, Jaeger). The best of all measurements of $FEV_1$ after bronchodilation was compared to predicted values, according to Tammeling and Quanjer.[267] The following groups were formed:

-Group I (asthma): patients with distinct asthmatic attacks, relatively symptom-free episodes and considerable reversibility of obstruction. Maximum $FEV_1$ was at least 80% of the predicted value.

-Group II (asthma + CAO): patients with asthmatic attacks at present or in the past, but with signs of irreversible chronic airflow obstruction (CAO) and/or chronic mucous hypersecretion at present. Maximum $FEV_1$ varied between 65% and 80% of the predicted value.

-Group III (CAO): subjects suffering from, usually severe, dyspnoea on exertion with only slight fluctuations, and distinct irreversible airflow obstruction, the best $FEV_1$ measured being less than 65% of the predicted value.

7-2-2 Group A - the "Senioren"

Originally, 126 patients of this group, no. A1 to A126, participated in the survey. Four patients were discharged from the clinic within 6 weeks after entrance to the study and were excluded, leaving 122 "Senioren" patients. There were 45 females and 77 males with an average age of 52 years (31-73 years). In Table 7-1 patients are classified according to sex and age.

Table 7-1 Group A patients, according to sex and age

| Age (yrs) | Male | Female | Total |
|---|---|---|---|
| 30-34 | 4 | 1 | 5 |
| 35-44 | 12 | 13 | 25 |
| 45-54 | 31 | 14 | 45 |
| 55-64 | 19 | 15 | 34 |
| 65-74 | 11 | 2 | 13 |
| Total | 77 | 45 | 122 |

With regard to classification according to clinical picture, as described in chapter 7-2-1, 56 patients were categorized into group I (asthma), 42 into group II (asthma + CAO) and 24 into group III (CAO). The most important characteristics of patients, according to clinical groups, are given in Table 7-2.

Table 7-2 Characteristics of group A patients, according to clinical picture

| | No. | Male/female | Age | Max.$FEV_1$/ predicted $FEV_1$ (%) |
|---|---|---|---|---|
| Group I | 56 | 29/27 | 49.5 | 99 |
| Group II | 42 | 28/14 | 52 | 69 |
| Group III | 24 | 20/ 4 | 57 | 45 |

7-2-3 Group B - the "Junioren"

Of the "Junioren" 79 patients were included in the study originally, no. B1 to B79. Seven patients dropped out because of discharge within 6 weeks and one patient was transferred to group A and is classified there. Forty-one male and 30 female patients remained, with an average age of 22 years (14-35 years). In Table 7-3 these patients are classified according to age and sex.

Table 7-3 Group B patients, according to sex and age

| Age (yrs) | Male | Female | Total |
|---|---|---|---|
| 14 | 2 | - | 2 |
| 15-19 | 20 | 10 | 30 |
| 20-24 | 9 | 12 | 21 |
| 25-29 | 5 | 4 | 9 |
| 30-35 | 5 | 4 | 9 |
| Total | 41 | 30 | 71 |

Although uncomplicated bronchial asthma with good reversibility was the common diagnosis here, to wit in 45 subjects, 23 had signs of asthma and irreversibility and 3 had features of progressive airflow obstruction without asthmatic attacks at present or in the past. Table 7-4 lists some characteristics of these patients.

Table 7-4 Characteristics of group B patients, according to clinical picture

| | No. | Male/female | Age | Max.$FEV_1$/ predicted $FEV_1$ (%) |
|---|---|---|---|---|
| Group I | 45 | 25/20 | 21 | 99 |
| Group II | 23 | 14/ 9 | 25 | 71 |
| Group III | 3 | 2/ 1 | 22 | 60 |

7-2-4 Group C - the "Vossen"

Group C comprised 23 boys and 9 girls, C1 to C 32, none of whom dropped out. Ages varied from 9 to 16 years with an average of 12 years. Thirty patients had bronchial asthma with strong allergic features, 4 of them having incomplete reversibility (group II). One girl with totally reversible asthmatic signs without allergic features and one other girl with cystic fibrosis were categorized into groups I and III, respectively. Thus the following score in clinical groups was achieved (Table 7-5).

Table 7-5 Characteristics of group C patients, according to clinical picture

| | No. | Male/female | Max.$FEV_1$/ predicted $FEV_1$ (%) |
|---|---|---|---|
| Group I | 27 | 19/8 | 105 |
| Group II | 4 | 4/- | 77 |
| Group III | 1 | -/1 | 61 |

7-2-5 Summary

Summarizing and combining the data presented above, we find a clear preponderance of patients with distinct asthmatic symptoms and complete reversibility of bronchial obstruction (group I). Subjects with the condition characterized by largely irreversible chronic airflow obstruction without paroxysmal dyspnoea (group III) are relatively scarce and for obvious reasons mainly to be found in group A. In Table 7-6 the different categories of patients are combined.

Table 7-6 Population of patients, according to living unit, clinical picture and sex

| | Group A "Senioren" | | Group B "Junioren" | | Group C "Vossen" | | Subtotal | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | |
| Group I asthma | 29 | 27 | 25 | 20 | 19 | 8 | 73 | 55 | 128 |
| Group II asthma + CAO | 28 | 14 | 14 | 9 | 4 | – | 46 | 23 | 69 |
| Group III CAO | 20 | 4 | 2 | 1 | – | 1 | 22 | 6 | 28 |
| Subtotal | 77 | 45 | 41 | 30 | 23 | 9 | 131 | 84 | |
| Total | 122 | | 71 | | 32 | | | | 225 |

7-3 Clinic

7-3-1 Respiratory infection criteria

Daily surveillance was carried out for each patient. The following symptoms were registered on a standard form:

-running nose

-stuffed nose

-sneezing

-sore throat

-sore ear(s)

-dry coughing

-hoarseness

-malaise.

A symptomatic respiratory infection (SRI) was supposed to be present if 2 or more of the mentioned symptoms turned out to be positive for 36 h or longer afer having been negative for the previous 4 days at least. In that case, coexisting symptoms like headache, myalgia, chest pain, itchy eyes, vomiting, and diarrhoea were noted, and physical examination of ears, throat and thorax daily as well as rectal temperature measurements twice daily were carried out.

7-3-2 Exacerbation criteria

Daily scores for dyspnoea, wheezing, coughing and sputum production were registered, using the following criteria:

-Dyspnoea. Grade 1: No dyspnoea.
Grade 2: Dyspnoea at heavy physical exertion only (walking uphill, climbing stairs).
Grade 3: Dyspnoea at moderate physical exertion (walking on flat terrain).
Grade 4: Dyspnoea at mild physical exertion (getting dressed).
Grade 5: Dyspnoea at rest.

-Wheezing. Grade 1: No wheezing.
Grade 2: Wheezing at physical exertion only.
Grade 3: Wheezing at rest.

-Coughing. Grade 1: No coughing.
Grade 2: Mild to moderate coughing.
Grade 3: Severe coughing or serious coughing spells.

-Sputum. Grade 1: 0-2 phlegms.
Grade 2: 3-5 phlegms; a: white-greyish
Grade 3: 6-9 phlegms; b: yellow-greenish
Grade 4: 10 or more phlegms c: green-purulent.

$FEV_1$ was measured daily at 5.30 p.m. on a hot wire spirometer, type Monaghan M403 (Sandoz) until October 1, 1977 and on a dry spirometer, type Vicatest (Mynhardt) thereafter. The better of 2 forced expiratory manoeuvres was noted.

Scores for dyspnoea/wheezing (combined) and cough/sputum (combined)

as well as $FEV_1$ in the course of an SRI were compared to the average of 10 scores preceeding the SRI. An exacerbation was defined as:
-An increase by at least 2 points in dyspnoea/wheezing or cough/sputum during 2 or more consecutive days.
-A decrease in $FEV_1$ by at least 25% during 2 or more consecutive days.
Whenever justified, we tried to keep medication constant.

7-3-3 Laboratory

On the second or third day of an SRI and 2-3 weeks afterwards a venous blood sample was taken. Furthermore, every 4 weeks blood samples were collected to check whether any subclinical infections had occurred. After centrifugation the sera were stored at $-20^{\circ}$ C. CF tests were done as described by Lennette[158], using the following antigens:
-Influenza A and B virus.
-Parainfluenza 1,2, and 3 virus.
-Adenovirus.
-RSV.
-*Mycoplasma pneumoniae*.
-*Chlamydia psittaci*.
Influenza A and B viruses were also tested by the HI method according to Masurel[173], using specific epidemic strains. A fourfold or greater rise in antibody titre was regarded as seroconversion. If green or yellow sputum was produced, patients were asked to collect it and a gram stain of a carefully washed flake was made according to the method of Mulder.[195] Bacteria in the adequate gram stain were considered as evidence of bacterial infection.

In the course of an SRI the following laboratory investigations were done, whenever possible, on the 2nd, 4th, 6th, and 10th day:
-Sedimentation rate of erythrocytes.
-Total leucocyte count and differentiation of leucocytes.
-Eosinophilic leucocyte counts.
Citrate for sedimentation rate and EDTA for eosinophilic leucocyte counts were added to venous blood samples, which were taken between 9 and 10 a.m. Subjects were not under basal conditions at the time of blood collection. Once in 4 weeks the laboratory procedures were

routinely performed in combination with blood sampling for regular serological investigations.

In case of SRI with severe symptoms and/or physical diagnostic signs of pulmonary infiltration, a frontal chest röntgenogram was made and compared to previous X-ray photographs. Furthermore, from January 1, 1977 until April 1, 1978 a röntgenogram of the thorax was a standard procedure in the course of an SRI.

7-4 Influenza vaccination

After detailed information with regard to influenza vaccination as well as the purposes of the study had been given to all participants, patients were left free to chose for or against vaccination. If urgent indication for vaccination was present in the opinion of the medical attendant, patients were strongly advised to have themselves vaccinated. Decisions with respect to vaccination in children were taken in consultation with their parents whenever possible.

# CHAPTER 8
# RESULTS FOR LIVING-GROUPS A, B, AND C

## 8-1 Introduction

From an epidemiological point of view it is of interest to look at the clinical and serological results in groups of patients divided according to living-units. Thus, in this chapter, a survey of the various periods of infection will be given separately for the groups A ("Senioren"), B ("Junioren"), and C ("Vossen"). Frequencies of respiratory infection are compared and effects on symptoms and signs of CNSLD will be estimated. The clinical picture of symptomatic respiratory infection (SRI), defined according to criteria mentioned in chapter 7-3-1, serves as a guideline throughout this chapter and the next ones. Furthermore, special attention will be given to periods in which seroconversion was found without obvious symptoms of respiratory infection.

## 8-2 Group A - the "Senioren"

### 8-2-1 Incidence of respiratory infection

As was described in chapter 7-2-2, 122 patients of group A took part in the study. The duration of participation varied from 1.5 to 17 months with an average of 5.9 months. Twelve persons participated in the course of 2 different periods, 5 in 3 periods. If a month during which one patient was under observation is considered as one patient month, there were 720.5 patient months in this group. A total of 127 SRIs were registered in the course of the survey, which is equivalent to approximately 0.18 SRI per patient month and 2.1 SRI per patient year. SRIs were more frequently observed during the months of December to April: 0.26 per patient month, compared to 0.12 during the remaining months. The number of SRIs per patient varied from 0 to 5 in accordance with differences in duration of participation (Table 8-1).

In 44 out of 127 episodes of SRI (34.7%) a viral agent was detected by means of serological investigations. In the remaining 83 SRIs no

agent could be identified. Seroconversions to the various agents in the course of 44 SRIs are listed in Table 8-2.

Table 8-1 No. of SRIs per patient and average study period. Group A patients.

| No. of SRIs | No. of patients | Average study period (months) |
|---|---|---|
| 2-5 | 34 | 8.2 |
| 1 | 40 | 6.9 |
| 0 | 48 | 4.7 |

Table 8-2 Agents involved in 44 periods of SRI, detected by fourfold or greater rise in antibody titre. Group A patients.

| Agent | No. of SRIs with seroconversion |
|---|---|
| Influenza A virus | 29 |
| RSV | 5 |
| Influenza B virus | 4 |
| Adenovirus | 3 |
| Parainfluenza 3 virus | 2 |
| Influenza A virus + RSV | 1* |
| Total | 44 |

*double infection

Fig. 8-1 gives a review of all SRIs experienced by group A patients in the course of the study.

In addition to the significant rises in titres found in SRI, 23 seroconversions were noted by means of routine investigations performed every 4 weeks. Agents involved are summarized in Table 8-3. In 15 of these cases the time of infection could be traced because of clinical symptoms too mild to exceed the threshold set for SRI, while in 6 seroconversions there were no symptoms of respiratory infection at all.

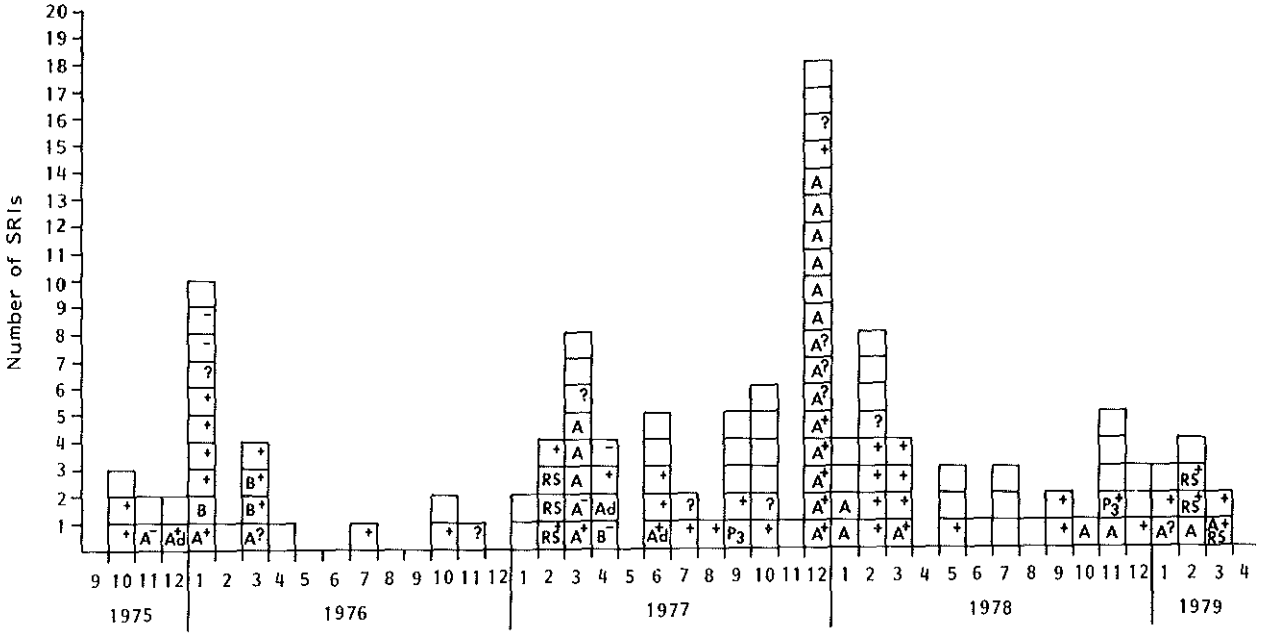


Fig. 8-1 Periods of SRI with results of serological and gram stain investigations in group A patients. A = influenza A; B = influenza B; Ad = adeno; RS = respiratory syncytial virus; $P_3$ = parainfluenza 3; + = positive sputum gram stain; - = negative sputum gram stain; ? = yellow/green sputum, no gram stain made.

Fig. 8-2 summarizes the 23 seroconversions without SRI, wheras Fig. 8-3 (Appendix II) shows all episodes of interest, experienced by the individual subjects in the periods in which they participated in the study.

According to clinical and serological data, three different groups were formed, the results of which will be discussed separately.

Table 8-3 No. of seroconversions to various agents, without the occurrence of SRI in group A patients

| Agent | No. of seroconversions |
|---|---|
| Influenza A virus | 10 |
| RSV | 9 |
| Influenza B virus | 2 |
| Parainfluenza 3 virus | 2 |
| Total | 23 |

8-2-2 SRI of proven viral origin

8-2-2-1 Clinical data

In the 44 periods of SRI with positive serology clinical features were usually distinct. The average amount of symptoms was somewhat over 6 and varied from 2 to 11. The minimum threshold of 2 symptoms was registered in 3 patients only. In 42 of these episodes data regarding body temperature were provided. Maximum values in the course of the infective illnesses are given in Table 8-4. The majority of SRIs (76%) were accompanied by fever, if a threshold value of 38° C is employed. On physical examination 14 patients showed marked signs of upper respiratory infection.

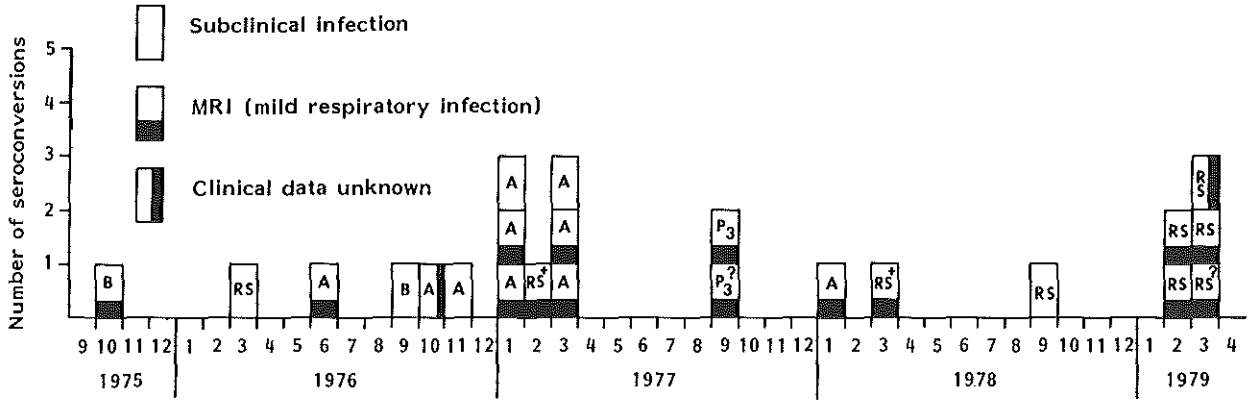


Fig. 8-2 Seroconversions without SRI in group A patients. A = influenza A; B = influenza B; RS = respiratory syncytial virus; $P_3$ = parainfluenza 3; + = positive sputum gram stain; ? = yellow/green sputum, no gram stain made

Table 8-4 Highest body temperatures in the course of SRI of viral origin in group A patients

| Maximum rectal temperature ($^{o}$ C) | No. of SRIs |
|---|---|
| <38 | 10 |
| 38-39 | 13 |
| 39-40 | 15 |
| ≥40 | 4 |
| Unknown | 2 |
| Total | 44 |

8-2-2-2 Effects on CNSLD parameters

In the course of 44 viral SRIs, exacerbation was noticed in 38 (86%). A bronchial obstructive pattern could be objectivied by means of daily $FEV_1$ measurements in a majority of patients. According to our standards (chapter 7-3-2), a decrease of $FEV_1$ was registered in 24 subjects. Twenty-nine patients complained of increased breathlessness and wheezing. Augmented productive coughing was also noticed in 29 SRIs. The various patterns of scores are summarized in Table 8-5.

Decrease in $FEV_1$ by 25% or more usually lasted for a relatively short period of 2-4 days. In 7 patients a reaction of 5 days or longer was noticed. One patient suffering from an adenovirus infection had prolonged symptoms of malaise and lassitude and his asthma was poorly controlled for several weeks, whereas a steady state existed before the SRI. Data of this patient are shown in case report 1 (Appendix I). Eight patients showed relatively severe bronchial obstructive reactions with $FEV_1$ decreasing to less than 50% of the baseline value. Two examples are given in case reports 2 and 3 (Appendix I).

Eight patients were removed to a special unit for more intensive medical care and in 11 subjects corticosteroid treatment was started or pre-infection steroid doses were raised.

Table 8-5 Patterns of exacerbation in the course of SRI with positive serology in group A patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 15 |
| + | + | - | 5 |
| + | - | + | 2 |
| + | - | - | 2 |
| - | + | + | 7 |
| - | + | - | 2 |
| - | - | + | 5 |
| - | - | - | 6 |
| | | | 44 |

8-2-2-3 Results of sputum gram stains

In the course of 25 SRIs with positive serology yellow or green sputum was produced. Material suitable for washing and gram staining was provided in 20 of these episodes. Seventeen of the gram preparations were assessed to be positive, whereas in 3 no bacteria were found. Eighteen of the patients produced coloured sputum and did not do so during the period preceeding the SRI. Two subjects developed a pneumonia by *Staphylococcus aureus* following influenza A infection. Antibiotic treatment was administered to 83% of the patients with yellow-green sputum, whereas 45% of the remaining subjects were also treated with antibiotics. Results of sputum gram stains are incorporated in Fig. 8-1 and in Appendix II, Fig. 8-3.

8-2-2-4 Results of laboratory investigations

Sedimentation rate, total leucocyte and eosinophilic leucocyte counts were made according to the programme in 34 out of the 44 episodes. Sedimentation rates showed a distinct increase of at least 15 mm in the first and/or second hour in 19 SRIs, usually in association with influenza A or bacterial superinfection. In general, total and eosino-

philic leucocyte counts did not seem to be of much value. Leucocytosis of over 10 x $10^9$/l was observed in 6 cases, whereas obvious leucopenia did not occur. Eosinophiles tended to decline in the course of SRI, especially when body temperature was high, but data were not consistent enough to draw conclusions.

Chest röntgenograms were made in 15 episodes of viral SRI. One of these showed multiple infiltrative changes associated with infection by adenovirus. The röntgenogram and relevant data are presented in case report 1 (Appendix I).

8-2-3 SRI without positive serology

8-2-3-1 Clinical data

In 83 periods of SRI no causative agent could be identified by means of serological tests. The clinical picture of respiratory infection was usually obvious, as in viral SRI, the average amount of symptoms being almost 7, varying between 2 and 12. Only 2 patients had the minimum of 2 symptoms. Rectal body temperature data were handed in by the patients in 78 instances. Maximum values were 38° C or more in the course of 42 SRIs (54%). Data are summarized in Table 8-6.

Table 8-6 Highest body temperature in the course of SRI without positive serology in group A patients

| Maximum rectal temperature (°C) | No. of SRIs |
|---|---|
| <38 | 36 |
| 38-39 | 24 |
| 39-40 | 17 |
| ≥40 | 1 |
| Unknown | 5 |
| Total | 83 |

8-2-3-2 Effects on CNSLD parameters

In the course of 2 SRIs without positive serology data were insufficient to draw any conclusions. In the remaining 81 an association with exacerbation of the obstructive disease was found in 53 (65%). Dyspnoea and wheezing increased in 38 patients and cough and sputum in 37, whereas significant decline of $FEV_1$ was noted in fewer patients, namely 25. Various combinations of parameters are shown in Table 8-7.

Table 8-7 Patterns of exacerbation in the course of SRI without positive serology in group A patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 12 |
| + | + | - | 7 |
| + | - | + | 2 |
| + | - | - | 4 |
| - | + | + | 14 |
| - | + | - | 5 |
| - | - | + | 9 |
| - | - | - | 28 |
| Inconclusive | Inconclusive | Inconclusive | 2 |
| | Total | | 83 |

Presence of fever did not influence the exacerbation rate: 23 out of 36 SRIs without fever (64%) and 26 out of 40 periods accompanied by fever (65%) were related to exacerbation.

Serious obstructive reactions with $FEV_1$ decreasing by over 50% were seen in 8 SRIs. One example of these in shown in case report 4 (Appendix I). Patients in whom increased bronchial obstruction could be confirmed by $FEV_1$ measurements usually had reductions of $FEV_1$ of short duration. Fifteen decreases in $FEV_1$ lasted for 2 or 3 days, whereas 4 prolonged reactions of at least 7 days were observed. In 2 subjects a stationary situation was thrown out of balance in the course of SRI. One of these, a 61-year-old asthmatic female patient, never returned to

her pre-infection condition and required significantly more bronchodilating drugs than before SRI. Some of the data of this patient are shown in case report 5 (Appendix I). In all other participants with exacerbations, symptoms subsided without problems and no long term effects were observed.

8-2-3-3 Results of sputum gram stains

Yellow or green sputum was produced in the course of 41 of the SRIs of unknown origin. Suitable samples were handed in by patients in 34 instances. Thirty-one of the gram-stained preparations were positive, whereas in 3 no bacteria were detected. Data are incorporated in Fig.8-1 and in Appendix II, Fig. 8-3.

On the base of clinical, bacteriological and laboratory findings an attempt was made to separate the SRIs that were thought to be of primarily bacterial origin. In 9 cases there were relatively few upper respiratory tract symptoms, considerable increases in sedimentation rate and/or leucocytosis. Yellow-greenish sputum was produced following a period of increased bronchial obstruction for other than infectious reasons in 6 of these SRIs. Two subjects showed the interesting phenomenon of increased bronchial obstruction following influenza vaccination, complicated by bacterial infection. One example is given in case report 6 (Appendix I).

Although bronchial obstruction usually preceeded infected sputum, $FEV_1$ tended to decrease further in a number of episodes. In 7 of the SRIs, presumably of bacterial origin, serious obstructive reactions with distinct effects on $FEV_1$ were noted. Case report 7 (Appendix I) shows one of these.

Antibiotics were given to 32 of the subjects producing yellow or green sputum. Furthermore, 9 of the remaining patients received antibiotic treatment.

8-2-3-4 Results of laboratory investigations

Investigations with regard to sedimentation rate, total and eosinophilic leucocyte counts were done at least twice in the course of 75 SRIs. As with SRI of viral origin, data were precarious and did not allow definite

conclusions.

Sedimentation rates showed increases of at least 15 mm in 20 periods, 8 of these being referred to as primarily bacterial. Leucocytosis of over 10 x $10^9$/l was noted in 11 episodes, 5 of which were considered to be of bacterial origin. On one occasion only, laboratory results were used as a diagnostic tool. In the course of an apparent respiratory infection a considerable increase of eosinophilic leucocytes was found, despite rectal body temperature of over 39° C. Moreover, pulmonary shadows were seen on the chest röntgenogram (see case report 8, Appendix I). These findings, together with relief of complaints at the institution of corticosteroids made the diagnosis eosinophilic pneumonia plausible.

Frontal röntgen photographs of the thorax were made in 42 periods of SRI. Infiltrative changes were found on 3 occasions, including the above mentioned episode of eosinophilic pneumonia.

8-2-4 Seroconversions without SRI

8-2-4-1 Clinical data

At the routine serological examinations 23 rises in titre were found, while in the relevant period no SRI occurred, according to our criteria. Fifteen subjects turned out to have experienced mild respiratory symptoms during the period in which seroconversion was noted. Usually only one symptom of infection was positive or two were, but had a very mild character. These episodes were named "mild respiratory infection" (MRI). One patient had slight malaise complaints associated with an elevated rectal body temperature of 39° C for one day. In 4 other subjects who measured body temperature, values did not exceed 38° C. Four persons produced yellow-green sputum in the course of MRI. Gram stains were made of 2 specimens, both of which were positive. Six infections passed off without clinical symptoms at all and were called "subclinical infections". In 2 patients clinical data were insufficiently noted, because they were not under observation at the relevant time.

Because SRI was not supposed to be present, laboratory and röntgenologic examinations were not undertaken in the majority of instances.

8-2-4-2 Effects on CNSLD parameters

The various scores for CNSLD parameters in 15 MRIs and 6 subclinical infections are summarized in Table 8-8. Subclinical infections did not cause any exacerbation of CNSLD, whereas MRI was associated with flare-ups in 6 out of 15 episodes (40%). Two patients had reactions in $FEV_1$ of at least 25% for 2 or more days, whereas another subject had decreases of 26 and 23% on 2 consecutive days, because of which corticosteroids were raised. On the base of retrospective data, exacerbation was suspected in both patients whose clinical data were unknown.

Table 8-8 Patterns of exacerbation in seroconversion without SRI in group A patients

| $FEV_1\downarrow$ | Dyspnoea/wheezing$\uparrow$ | Cough/sputum$\uparrow$ | MRI | Subclinical |
|---|---|---|---|---|
| + | + | + | 1 | - |
| + | + | - | - | - |
| + | - | + | - | - |
| + | - | - | 1 | - |
| - | + | + | 1 | - |
| - | + | - | - | - |
| - | - | + | 3 | - |
| - | - | - | 9 | 6 |
| | | Total | 15 | 6 |

8-3 Group B - the "Junioren"

8-3-1 Incidence of respiratory infection

Of group B, 71 patients participated in the study (see chapter 7-2-2 for details). The average study period for these subjects was 8.7 months, varying from 1.5 to 20.5 months. Six patients were involved in the survey twice. There were a total of 616.5 patient months, in which 98 SRIs occurred, yielding a frequency of 0.16 SRI per patient month and of 1.9 SRI per patient year. The frequency in the months of December, January, February, and March was 0.22 compared to 0.13 per patient month in the remaining months. The number of symptomatic infections per person ranged

from 0 to 4, related to duration of participation in the study, as is shown in Table 8-9.

Table 8-9 No. of SRIs per patient and average study period. Group B patients

| No. of SRIs | No. of patients | Average study period (months) |
|---|---|---|
| 2-4 | 30 | 10.2 |
| 1 | 26 | 8.9 |
| 0 | 15 | 5.3 |

In 27 out of 98 SRIs (28%) significant rises in titre were detected by means of serological investigations; only 2 different agents were involved, as shown in Table 8-10. A survey of all episodes of SRI is given in Fig. 8-4.

Table 8-10 Agents involved in 27 periods of SRI, detected by fourfold or greater rise in antibody titre. Group B patients

| Agent | No. of SRIs with seroconversion |
|---|---|
| Influenza A virus | 21 |
| Parainfluenza 3 virus | 6 |
| Total | 27 |

In addition to the seroconversions found in SRI, 23 significant rises in antibodies to various agents, including 2 double ones were noted at the routine serological investigations. Results are shown in Table 8-11.

In 12 of these patients an MRI could be shown to have occurred, whereas 7 subclinical infections were noted. In 2 periods clinical data were insufficiently collected, because subjects were out of observation. Data are summarized in Fig. 8-5. A survey of all relevant episodes per subject is given in Fig. 8-6 (Appendix II).

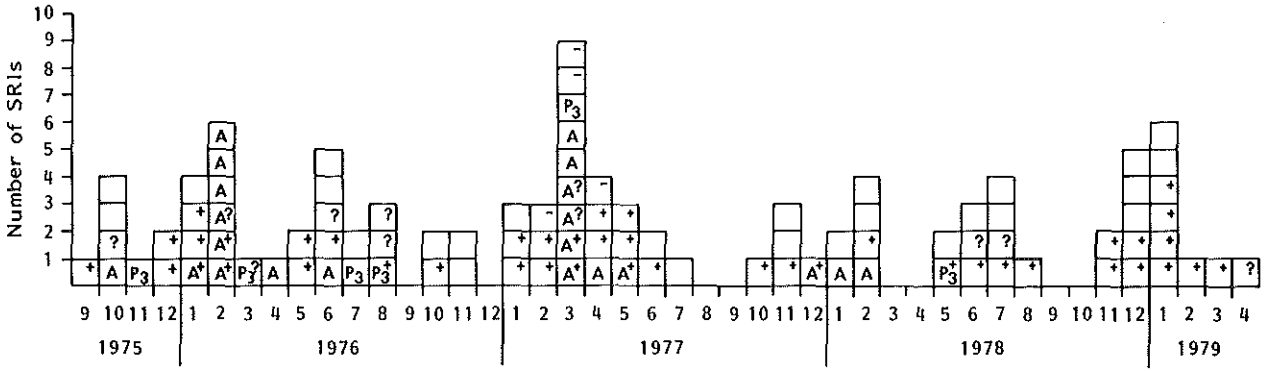


Fig. 8-4 Periods of SRI with results of serological and gram stain investigations in group B patients. A = influenza A; $P_3$ = parainfluenza 3; + = positive sputum gram stain; − = negative sputum gram stain; ? = yellow/green sputum, no gram stain made

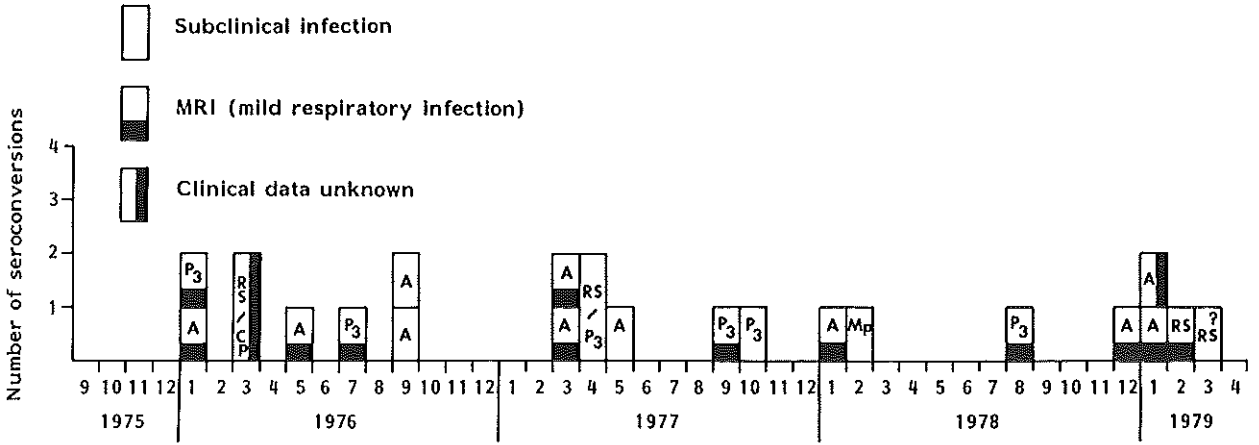


Fig. 8-5 Seroconversions without SRI in group B patients. A = influenza A; RS = respiratory syncytial virus; $P_3$ = parainfluenza 3 virus; Mp = *Mycoplasma pneumoniae*; Cp = *Chlamydia psittaci*; ? = yellow/green sputum, no gram stain made

Table 8-11 No. of seroconversions to various agents, without the occurrence of SRI in group B patients

| Agent | No. of seroconversions |
|---|---|
| Influenza A virus | 11 |
| Parainfluenza 3 virus | 5 |
| RSV | 2 |
| *Mycoplasma pneumoniae* | 1 |
| RSV + *Chlamydia psittaci* | 1* |
| RSV + parainfluenza 3 virus | 1* |
| Total | 23 |

*double infection

8-3-2 SRI of proven viral origin

8-3-2-1 Clinical data

In the course of the SRIs with positive serology, clinical features were in general distinct but slightly milder than in group A. An average amount of 5 symptoms were present, varying from 2 to 10. The minimum of 2 symptoms were noted once. Twenty-four patients measured rectal body temperature and 18 of them (75%) showed values of at least 38° C. Data are shown in Table 8-12. On physical examination 11 subjects showed marked signs of upper respiratory tract infection.

Table 8-12 Highest body temperatures in the course of SRI of viral origin in group B patients

| Maximum rectal temperature (° C) | No. of SRIs |
|---|---|
| <38 | 6 |
| 38-39 | 7 |
| 39-40 | 9 |
| ≥40 | 2 |
| Unknown | 3 |
| Total | 27 |

8-3-2-2 Effects on CNSLD parameters

Of 27 infections with positive serology 20 (74%) were associated with exacerbation, according to the criteria mentioned in chapter 7-3-2. Distinct bronchoconstrictive patterns were frequently noted: 17 subjects had decreases in $FEV_1$, but only 11 of them complained of increased dyspnoea and wheezing. Increase in productive coughing was observed in 14 SRIs and was the only factor accounting for exacerbation in 3 of these. Detailed data are presented in Table 8-13.

Table 8-13 Patterns of exacerbation in the course of SRI with positive serology in group B patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 7 |
| + | + | - | 4 |
| + | - | + | 4 |
| + | - | - | 2 |
| - | + | + | - |
| - | + | - | - |
| - | - | + | 3 |
| - | - | - | 7 |
| | | Total | 27 |

Obstructive reactions were relatively severe with $FEV_1$ decreasing by over 50% in 8 subjects. Two examples are given in case reports 9 and 10 (Appendix I). In 10 SRIs $FEV_1$ returned to baseline values within 4 days, whereas 7 reactions of at least 5 days were observed. Two prolonged reactions were seen in the course of parainfluenza 3 infections (see case report 11, Appendix I, for one example), but no real long-term effects were noted.

Two patients suffering from influenza A infection were taken to a special care station, whereas one patient, in spite of medical instructions, travelled to Holland in the course of an influenza A infection and was admitted to hospital there. To one subject a single dosé of

tetracosactide depot was administered and in 3 patients who were on corticosteroids, doses had to be raised.

8-3-2-3 Results of sputum gram stains

Coloured sputum, suspected of bacterial infection, was coughed up by 13 patients in the course of an SRI. Nine sputum samples were handed in and gram stained, and all of these turned out to be positive (Fig. 8-4 and Appendix II, Fig. 8-6). In the remaining 4 cases, sputum was either not collected or unsuitable for preparation. Increased obstruction was frequently associated with bacterial infection, namely in 11 instances. Changes in sputum colour were preceeded by obstruction in 7 patients, one of whom is described in case report 12 (Appendix I). Six patients received antibiotic treatment.

8-3-2-4 Results of laboratory investigations

Laboratory parameters were sufficiently established in 22 patients. Eleven of them showed increases of sedimentation rates of over 15 mm, whereas leucocytosis of over 10 x $10^9$/l was observed in 5 subjects. Marked eosinopenia in the course of SRI was noted in 10 patients and occurred in the presence of fever only. No further correlations between clinical data and laboratory investigations were found. Chest röntgenograms were manufactured of 6 patients. No infiltrative changes were observed.

8-3-3 SRI without positive serology

8-3-3-1 Clinical data

SRIs in which serology remained negative were, as in group A, usually associated with marked symptomatology. An average of over 6 symptoms were noted, the minimum of 2 being registered only twice. Sixty-seven subjects presented body temperature data; of these patients 41 (61%) had maximum values of at least 38° C. Table 8-14 summarizes results of rectal temperatures.

Table 8-14 Highest body temperatures in the course of SRI without positive serology in group B patients

| Maximum rectal temperature (° C) | No. of SRIs |
|---|---|
| <38 | 26 |
| 38-39 | 31 |
| 39-40 | 9 |
| ≥40 | 1 |
| Unknown | 4 |
| Total | 71 |

8-3-3-2 Effects on CNSLD parameters

A considerable part of SRIs could be related to exacerbation of CNSLD. A total of 50 flare-ups were observed in the course of 71 episodes of SRI (70%). Exacerbation was associated with decreasing $FEV_1$ in 27 instances, whereas increases in dyspnoea/wheezing and cough/sputum were reported by 28 and 32 subjects, respectively. Augmentation of cough and sputum alone accounted for 13 exacerbations. Data are listed in Table 8-15.

Table 8-15 Patterns of exacerbation in the course of SRI without positive serology in group B patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 11 |
| + | + | - | 7 |
| + | - | + | 5 |
| + | - | - | 4 |
| - | + | + | 3 |
| - | + | - | 7 |
| - | - | + | 13 |
| - | - | - | 21 |
| | Total | | 71 |

Fever was not related to exacerbation; of 26 SRIs without fever, 18 (69%) were associated with exacerbation, whereas this was true for 28 out of 41 SRIs (68%) with body temperatures of over 38° C. Severe asthmatic reactions were frequently observed. During 15 SRIs, $FEV_1$ decreased by 50% or more, no matter whether the reaction was of short, intermediate, or long duration. Examples are given in case reports 13 and 14 (Appendix I).

Reactions lasted for 2 or 3 days in 13 subjects, for 4 or 5 days in 5, and for one week or longer in 9. One female patient showed a prolonged reaction of a few weeks, but finally $FEV_1$ returned to pre-infection levels (case report 15, Appendix I).

8-3-3-3 Results of sputum gram stains

Forty-six subjects notified that yellow or green sputum was produced in the course of SRI. Acceptable samples were obtained in 39 cases, the gram stains of which were positive in 35 and negative in 4. Data are incorporated in Fig. 8-4 and in Appendix II, Fig. 8-6.

As in group A, an attempt was made to select periods that were probably due to bacterial infection alone. Using clinical, bacteriological, and laboratory data, 9 episodes were supposed to be of bacterial origin without being triggered by unidentified viral agents. Again it was found that bacterial infection frequently occurred following periods of increased bronchial obstruction, be it related to possible viral infections or not. Eighteen of the periods with yellow-green sputum were preceeded by augmented obstruction. Case report 16 (Appendix I) shows an example. Sixteen patients received antibiotic treatment in the course of SRI without positive serology.

8-3-3-4 Results of laboratory investigations

Laboratory results could be sufficiently obtained in 59 patients. Increases in sedimentation rate and leucocytosis were observed in 21 and 15 subjects, respectively, usually in association with bacterial sputum infection. Marked reduction in eosinophilic leucocytes occurred in 6 SRIs only.

Röntgen photographs of the chest were made in 36 instances, one of

which revealed pulmonary infiltrative abnormalities .

8-3-4 Seroconversions without SRI

8-3-4-1 Clinical data

As reported in chapter 8-3-1 seroconversion to various agents without the occurrence of SRI was detected on 23 occasions, including 2 double rises in titre. MRI was notified in 12 subjects. Symptoms were always too few and/or too short-lasting to exceed the threshold, set for SRI. One patient had a rectal body temperature of 38° C, all other subjects did not supply data with regard to fever.

Seven infections were regarded as subclinical because of the absence of any upper respiratory symptoms, whereas in 2 patients clinical data remained unknown, as they were not under observation in the relevant period.

8-3-4-2 Effects on CNSLD parameters

Combinations of scores for MRI and subclinical infections are presented in Table 8-16. Five out of 12 MRIs (42%) were related to exacerbation, 4 of these in association with changes in $FEV_1$. Two distinct asthmatic reactions in the course of influenza A and parainfluenza 3 infection

Table 8-16 Patterns of exacerbation in seroconversion without SRI in group B patients

| $FEV_1$ | Dyspnoea/wheezing | Cough/sputum | MRI | Subclinical |
|---|---|---|---|---|
| + | + | + | | |
| + | + | − | 1 | |
| + | − | + | 1 | |
| + | − | − | 2 | |
| − | + | + | | 1 |
| − | + | − | | |
| − | − | + | 1 | 1 |
| − | − | − | 7 | 5 |
| | | Total | 12 | 7 |

are shown in case reports 17 and 18 (Appendix I), respectively. Long-term effects were not noted.

Subclinical infection connected with exacerbation was observed twice (29%). *Mycoplasma pneumoniae* and RSV were associated with productive coughing, the latter of green sputum, of which no gram stain was made. The patient with seroconversion to RSV had a slight increase of obstruction as well, but decline in $FEV_1$ was insufficient to exceed threshold value, namely 16 and 27% on 2 consecutive days.

8-4 Group C - the "Vossen" *

8-4-1 Incidence of respiratory infection

Of the 32 children belonging to group C, the average duration of participation in the study was 14.5 months, varying from 3.5 to 30.5 months. None of the subjects took part in the study more than once. A total of 465 patient months was noted, in which 59 SRIs occurred. This is equivalent to a respiratory infection frequency of 0.13 per patient month and 1.5 per patient year. SRI was only slightly more frequent in the months of December to April: 0.15 per patient month, compared to 0.11 during the remaining months.

The number of infection periods per subject varied strongly, namely from 0 to 7, and was related to the duration of the study period, as is shown in Table 8-17.

Table 8-17 No. of SRIs per patient and average study period. Group C patients

| No. of SRIs | No. of patients | Average study period (months) |
|---|---|---|
| 4-7 | 4 | 27.5 |
| 2-3 | 13 | 17.5 |
| 0-1 | 15 | 8.5 |

*Part of this chapter will be published in the European Journal of Respiratory Diseases (A.C.Roldaan & N.Masurel)

In 19 out of 59 SRIs (32%) a viral causative agent was detected serologically. Of the remaining 40 episodes a pharyngitis by β-haemolytic group A streptococci was noticed in 5, whereas in 35 no agent could be identified. Various viral agents and *Mycoplasma pneumoniae* detected in the course of SRI are listed in Table 8-18.

Furthermore, 29 periods with a total of 31 seroconversions were found in routine serological investigations, performed every 4 weeks. The agents involved are presented in Table 8-19.

Table 8-18 Agents involved in 19 periods of SRI, detected by fourfold or greater rise in antibody titre. Group C patients

| Agent | No. of SRIs with seroconversion |
|---|---|
| Influenza A virus | 13 |
| Influenza B virus | 3 |
| Parainfluenza 1 virus | 1 |
| RSV | 1 |
| *Mycoplasma pneumoniae* | 1 |
| Total | 19 |

Table 8-19 No. of seroconversions to various agents, without the occurrence of SRI. Group C patients

| Agent | No. of seroconversions |
|---|---|
| Influenza A virus | 10 |
| Parainfluenza 3 virus | 8 |
| Influenza B virus | 4 |
| Adenovirus | 2 |
| RSV | 2 |
| *Chlamydia psittaci* | 1 |
| *M.pneumoniae* + parainfluenza 3 virus | 1* |
| Influenza A virus + RSV | 1* |
| Total | 31 |

*double infection

On 12 occasions mild respiratory symptoms were shown to have occurred, whereas 9 infections passed off without any symptoms. Ten rises in antibody titre, including the 2 double ones, were noted in periods during which children were not under observation because they spent their school holidays with their families. Fig. 8-7 and Fig. 8-8 show SRIs and seroconversions without SRI, respectively, whereas Fig. 8-9 (Appendix II) lists all relevant episodes per subject.

8-4-2 SRI of proven viral origin

8-4-2-1 Clinical data

During the 19 periods of SRI with positive serology, clinical features again were generally distinct. The minimum of 2 symptoms was found only twice and the average amount of symptoms was 6. Eighteen subjects registered body temperature correctly, with the following results (Table 8-20).

Table 8-20 Highest body temperatures in the course of SRI of viral origin in group C patients

| Maximum rectal temperature (° C) | No. of SRIs |
|---|---|
| <38 | 0 |
| 38-39 | 6 |
| 39-40 | 8 |
| ≥40 | 4 |
| Unknown | 1 |
| Total | 19 |

All children had fever in the course of viral SRI. Nine patients had distinct signs of upper respiratory tract infection on physical examination.

8-4-2-2 Effects on CNSLD parameters

Of all viral SRIs in this group, in 1 influenza B infection data were insufficiently collected to draw any conclusions. The remaining 18 episodes were associated with exacerbation in 16 instances (89%). Twelve

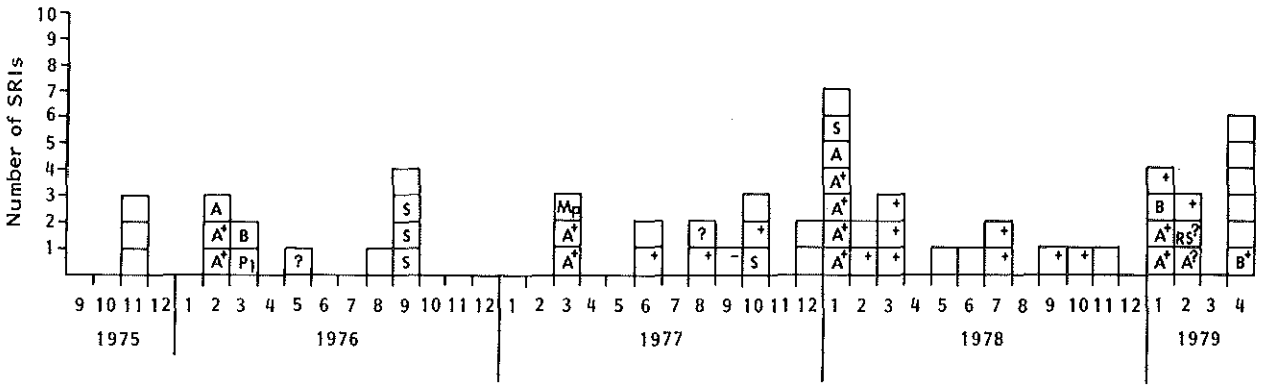


Fig. 8-7 Periods of SRI with results of serological and gram stain investigations in group C patients. A = influenza A; B = influenza B; RS = respiratory syncytial virus; $P_1$ = parainfluenza 1; Mp = *Mycoplasma pneumoniae*; S = β haemolytic group A streptococci; + = positive sputum gram stain; − = negative sputum gram stain; ? = yellow/green sputum, no gram stain made.

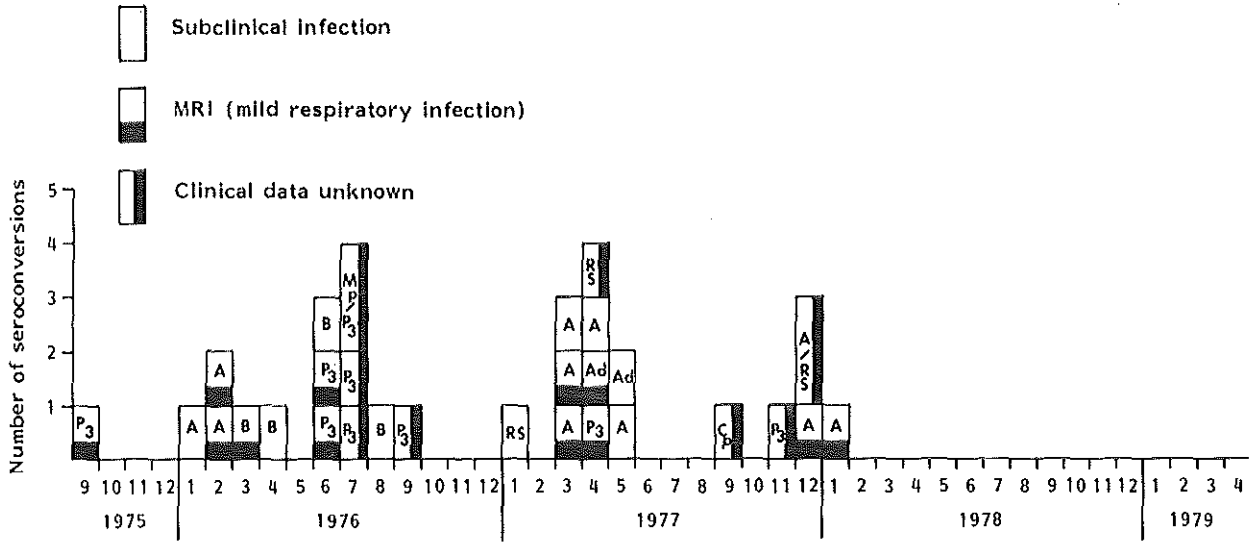


Fig. 8-8 Seroconversions without SRI in group C patients. A = influenza A; B = influenza B; Ad = adeno; RS = respiratory syncytial virus; $P_3$ = parainfluenza 3; Mp = *Mycoplasma pneumoniae*; Cp = *Chlamydia psittaci*

patients showed significant decreases in $FEV_1$, whereas only 7 complained of increased dyspnoea and wheezing. Furthermore, 12 subjects experienced augmented scores for cough/sputum. Data are summarized in Table 8-21.

Table 8-21 Patterns of exacerbation in the course of SRI with positive serology in group C patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 4 |
| + | + | - | 2 |
| + | - | + | 4 |
| + | - | - | 2 |
| - | + | + | 1 |
| - | + | - | - |
| - | - | + | 3 |
| - | - | - | 2 |
| | Inconclusive | | 1 |
| | Total | | 19 |

Serious asthmatic attacks accompanied by decrease in $FEV_1$ of over 50% were noticed in 9 patients. Some striking examples are shown in case reports 19 and 20 (Appendix I). In 6 children $FEV_1$ was reduced by at least 25% for 2 or 3 days, whereas in the remaining 6 the reactions lasted from 5-9 days. The most persistent reaction was seen in the patient suffering from *Mycoplasma pneumoniae* infection, who had only minor upper respiratory complaints (case report 21, Appendix I). Long-term effects were not observed in any of the episodes. In 2 patients ACTH or corticosteroids had to be given in the course of 4 SRIs.

8-4-2-3 Results of sputum gram stains

In the course of 13 episodes yellow or green sputum was produced. Samples could be obtained in 11 of these, the gram stains of which all turned out to be positive. Results are incorporated in Fig. 8-7 and in Appendix II, Fig. 8-9.

Positive gram stains were associated with obstruction on 8 occasions. One patient with bronchiectatic deformations in his middle and right upper lobe repeatedly developed severe pulmonary infiltrations in viral infection as well as in SRI without positive serology. One of these episodes is shown in case report 22 (Appendix I).

Antibiotic treatment was started in the course of 9 SRIs, including tetracyclins in the *Mycoplasma pneumoniae* infection, although sputum was not coloured.

8-4-2-4 Results of laboratory investigations

Laboratory data could satisfactorily be collected in 15 instances. While leucocytosis of over 10 x $10^9$/l was infrequent, namely in 2 patients, increased sedimentation rates and marked falls in eosinophilic leucocytes were observed in 12 and 11 patients, respectively.

Chest röntgenograms were made in 9 SRIs. The above mentioned boy had severe infiltrative changes during 3 periods of viral SRI, whereas röntgenograms were negative in the remaining instances.

8-4-3 SRI without positive serology

8-4-3-1 Clinical data

Forty episodes of SRI occurred in which serological investigations did not show any rise in titre. Symptoms of SRI were again obvious, with an average amount of 6, ranging from 3 to 11. Body temperature was recorded on 37 occasions. Data are given in Table 8-22.

Table 8-22 Highest body temperature in the course of SRI without positive serology in group C patients

| Maximum rectal temperature (°C) | No. of SRIs |
|---|---|
| <38 | 10 |
| 38-39 | 15 |
| 39-40 | 10 |
| ≥40 | 2 |
| Unknown | 3 |
| Total | 40 |

Fever was present in 27 subjects (73%). Five children presented with symptoms and signs of serious pharyngitis, accompanied by pus in tonsillar crypts and enlargement of cervical lymphnodes. Body temperatures ranged from 37.9° to 39° C. Bacteriological investigations yielded positive results for β-haemolytic group A streptococci.

8-4-3-2 Effects on CNSLD parameters

Before rating the scores for CNSLD, SRIs by haemolytic streptococci were separated. None of the streptococcal pharyngitis episodes caused any asthmatic reaction and $FEV_1$ values were remarkably consistent. Case report 23 (Appendix I) shows one of these instances.

In the remaining 35 SRIs exacerbation was noted in 23 (66%). Fourteen significant declines of $FEV_1$ were observed. Thirteen patients complained of increased breathlessness and wheezing, whereas 18 children mentioned augmented coughing and sputum production. Table 8-23 summarizes these results.

Table 8-23 Patterns of exacerbation in the course of SRI without positive serology in group C patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 7 |
| + | + | - | 3 |
| + | - | + | 3 |
| + | - | - | 1 |
| - | + | + | 2 |
| - | + | - | 1 |
| - | - | + | 6 |
| - | - | - | 12 |
| | Total | | 35 |

Eight severe reactions with $FEV_1$ decreasing to less than 50% of the baseline values were noted. Case report 24 (Appendix I) shows one of these. Obstructive exacerbations usually were of short duration: $FEV_1$

returned to pre-infection levels within 4 days in 10 children, whereas reactions of about one week were observed 4 times.

8-4-3-3 Results of sputum gram stains

Sixteen periods were associated with yellow or green sputum. Suitable material was handed in by 14 children. Thirteen gram stains came out positive. Infections were judged to be of bacterial origin solely in 6 instances. Four of these were preceeded by periods of increased asthmatic symptoms (see case report 25, Appendix I, for an example).

Results of gram preparations are incorporated in Fig. 8-7 and in Appendix II, Fig. 8-9.

8-4-3-4 Results of laboratory investigations

In case of streptococcal pharyngitis, SRI was associated with remarkable increases in erythrocyte sedimentation rates and leucocytosis in all subjects. Two patients with streptococcal infections showed distinct declines in eosinophilic leucocytes.

Of the remaining 35 SRIs data could be collected according to programme in 32. Ten children had increases of over 15 mm in sedimentation rate, whereas leucocytosis was observed in 4 and eosinopenia in 5 subjects. Data were suggestive for leucopenia in some patients, but the results were not consistent enough to draw conclusions.

Röntgen photographs of the chest were made in the course of 20 SRIs. The boy with repeated infiltrations showed a marked pulmonary shadow again on one occasion, all other X-rays were without abnormalities.

8-4-4 Seroconversions without SRI

8-4-4-1 Clinical data

Out of 31 seroconversions detected by routine serological examinations, 12 were related to MRI. Symptoms were too few or too short-lasting to exceed the threshold set for SRI in all but one patient, who on reviewing clinical data could have been regarded as having SRI, but 2 symptoms were present in a mild degree only. Another child had unexplained fever without obvious respiratory symptoms and turned out to have been infected by influenza A virus. Nine infections occurred without any

symptoms. In the remaining 8 episodes of 10 seroconversions (2 double ones were noted), patients spent their holidays outside the clinic, usually in Holland. Retrospective anamnestic data revealed features of SRI in 3 of them and were negative in this respect in 2, whereas doubtful history was given in the remaining subjects.

8-4-4-2 Effects on CNSLD parameters

Scores for MRI and subclinical infections are given in Table 8-24.

Table 8-24 Patterns of exacerbation in seroconversion without SRI in group C patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | MRI | Subclinical |
|---|---|---|---|---|
| + | + | + | – | 1 |
| + | + | – | 1 | – |
| + | – | + | – | – |
| + | – | – | 2 | 1 |
| – | + | + | – | – |
| – | + | – | – | – |
| – | – | + | 1 | – |
| – | – | – | 8 | 7 |
| | | Total | 12 | 9 |

Four exacerbations occurred in the course of 12 MRIs (33%), 2 of these associated with serious asthmatic reactions. Malaise was present, but any upper respiratory symptoms were not noted. Influenza A and adenovirus were detected serologically (case reports 26 and 27, Appendix I). Asthmatic reactions also occurred in 2 seroconversions in which symptoms were completely absent (22%). One interesting example of subclinical influenza A infection associated with severe asthmatic signs is given in case report 28 (Appendix I).

Although data were hardly registered during school vacations, when children were out of observation, retrospective information was useful in some instances. In the course of 8 outclinic episodes related to

seroconversion, 5 exacerbations were reported with certainty (63%). In 2 of these anamnestic data could be confirmed by $FEV_1$ measurements, which were made accidentally. Data are shown in Table 8-25 as complete as possible.

Table 8-25 Anamnestic data in the course of seroconversion in group C patients during outclinic periods

| Infection with | SRI | Exacerbation |
|---|---|---|
| Parainfluenza 3 virus | ? | + |
| Parainfluenza 3 virus | ? | – |
| Parainfluenza 3 virus | – | – |
| Influenza A virus + RSV | + | + |
| *M.pneum.* + parainfl.3 virus | + | + ($FEV_1\downarrow$) |
| RSV | ? | + |
| *Chlamydia psittaci* | + | + ($FEV_1\downarrow$) |
| Parainfluenza 3 virus | – | – |

8-5 Combination of data

If data are combined, the following results are obtained:

1. A total number of 225 CNSLD patients were followed for 1802 patient months.
2. Subjects experienced 284 periods of SRI, defined according to criteria, as described in chapter 7-3-1. This stands for an SRI frequency of 0.16 per patient month and 1.9 per patient year.
3. In the course of 90 out of 284 SRIs (32%) significant rises in antibody titre to various viral agents as well as *Mycoplasma pneumoniae* were detected.
4. Routine serological investigations performed every 4 weeks revealed another 81 seroconversions including 4 double ones. Of these, 39 could be related to MRI, whereas 22 were regarded as subclinical infections. The remaining conversions occurred while the relevant subjects were not under observation.
5. SRI of proven viral origin was associated with exacerbation of CNSLD, defined according to criteria in chapter 7-3-2, in 74 out of 89

periods (83%) in which data could be collected satisfactorily.

6. SRI without positive serology was related to exacerbation in 126 out of 192 episodes (66%).
7. In the course of seroconversion in which MRI occurred, exacerbation was noted on 15 out of 39 occasions (38%), whereas flare-ups were observed in 4 out of 22 periods of subclinical infection (18%).
8. Features were various with regard to exacerbation. Different combinations of increase in dyspnoea/wheezing and cough/sputum and/or decline in $FEV_1$ were observed. Results are summarized in Tables 8-26, 8-27, and 8-28.

Table 8-26 Patterns of exacerbation in the course of SRI with positive serology. All patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 26 |
| + | + | - | 11 |
| + | - | + | 10 |
| + | - | - | 6 |
| - | + | + | 8 |
| - | + | - | 2 |
| - | - | + | 11 |
| - | - | - | 15 |
| | Inconclusive | | 1 |
| | Total | | 90 |

Table 8-27 Patterns of exacerbation in the course of SRI without positive serology. All patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 30 |
| + | + | – | 17 |
| + | – | + | 10 |
| + | – | – | 9 |
| – | + | + | 19 |
| – | + | – | 13 |
| – | – | + | 28 |
| – | – | – | 66 |
| | Inconclusive | | 2 |
| | Total | | 194 |

Table 8-28 Patterns of exacerbation in seroconversion without SRI. All patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | MRI | Subclinical |
|---|---|---|---|---|
| + | + | + | 1 | 1 |
| + | + | – | 2 | – |
| + | – | + | 1 | – |
| + | – | – | 5 | 1 |
| – | + | + | 1 | 1 |
| – | + | – | – | – |
| – | – | + | 5 | 1 |
| – | – | – | 24 | 18 |
| | | Total | 39 | 22 |

# CHAPTER 9
# RESULTS FOR DIFFERENT CLINICAL GROUPS I, II, AND III

## 9-1 Introduction

In order to investigate whether differences with regard to respiratory infection exist between the various subpopulations of patients with CNSLD, the results were analysed again after dividing the study group in 3 subgroups according to clinical picture. The criteria of classification are given in chapter 7-2-1. In this chapter special attention will be given to the incidence of respiratory infection, the effects on CNSLD parameters, and the occurrence of bacterial sputum infection. The results are presented for group I (asthma), group II (asthma and chronic airflow obstruction (CAO)), and group III (CAO) separately and compared afterwards.

## 9-2 Group I - Asthma

### 9-2-1 Incidence of respiratory infection

By far most patients of the population studied were classified into group I, i.e. 128. These subjects participated in the study for a total of 1066 patient months, which stands for an average of 8.3 months per person.

A total of 158 periods of SRI were noted, yielding an incidence of 0.15 SRI per patient month for this group. Forty-five episodes of SRI were accompanied by significant rises in antibody titre to one or more of the agents tested (28%). The agents involved are described in Table 9-1.

In the remaining 113 episodes, serology remained negative. In addition, 51 seroconversions including 3 double ones, were detected in group I patients without the occurrence of SRI or in the absence of known clinical data. This equalizes to 0.05 seroconversions without SRI per patient month. In 24 instances seroconversion could be related to mild respiratory infection (MRI), whereas 15 subclinical infections and

9 periods of unknown clinical data were noted. The agents detected by routine serology are listed in Table 9-2.

Table 9-1 Agents involved in 45 periods of SRI detected by fourfold or greater rise in antibody titre. Group I patients

| Agent | No. of SRIs with seroconversion |
|---|---|
| Influenza A virus | 33 |
| Influenza B virus | 5 |
| Parainfluenza 3 virus | 4 |
| Parainfluenza 1 virus | 1 |
| RSV | 1 |
| *Mycoplasma pneumoniae* | 1 |
| Total | 45 |

Table 9-2 No. of seroconversions to various agents without the occurrence of SRI in group I patients

| Agent | No. of seroconversions | | | |
|---|---|---|---|---|
| | MRI | Subclinical infection | Clinical data unknown | Total |
| Influenza A virus | 11 | 7 | 2 | 20 |
| Parainfluenza 3 virus | 6 | 1 | 4 | 11 |
| RSV | 5 | 2 | 1 | 8 |
| Influenza B virus | 1 | 3 | – | 4 |
| Adenovirus | 1 | 1 | – | 2 |
| RSV + parainfl.3 virus | – | 1* | – | 1* |
| RSV + *Chlam.psittaci* | – | – | 1* | 1* |
| *M.pneum.* + parainfl.3 | – | – | 1* | 1* |
| Total | 24 | 16 | 11 | 51 |

*double infection

9-2-2 Effects on CNSLD parameters

9-2-2-1 SRI with positive serology

Of the 45 relevant periods, data with regard to the course of CNSLD could be collected satisfactorily in 44. Exacerbation was noted in 37 instances (84%). Significant decrease in $FEV_1$ was observed in 32 out of 44 episodes (73%). Augmentation of dyspnoea/wheezing and cough/sputum was registered in 20 (45%) and 25 (57%) periods, respectively. Different combinations are shown in Table 9-3.

Table 9-3 Patterns of exacerbation in the course of SRI with positive serology in group I patients

| $FEV_1\downarrow$ | Dyspnoea/wheezing$\uparrow$ | Cough/sputum$\uparrow$ | |
|---|---|---|---|
| + | + | + | 11 |
| + | + | − | 7 |
| + | − | + | 9 |
| + | − | − | 5 |
| − | + | + | 2 |
| − | + | − | − |
| − | − | + | 3 |
| − | − | − | 7 |
| | | Unknown | 1 |
| | | Total | 45 |

Severe bronchial obstructive reactions with $FEV_1$ decreasing by over 50% were observed in the course of 19 of the 44 SRIs (43%). Changes of at least 25% lasted 4 days or less on 21 and 5 days or more on 11 occasions.

9-2-2-2 SRI without positive serology

Out of the 113 episodes of SRI in which serological investigations were negative, data could be sufficiently collected in 111. Exacerbation was registered on 77 occasions (69%), with decrease of $FEV_1$ in 46 (41%), increase in dyspnoea/wheezing in 47 (42%), and augmentation of cough/sputum in 53 (48%) subjects. The various patterns of exacerbation are

summarized in Table 9-4.

Table 9-4 Patterns of exacerbation in the course of SRI without positive serology in group I patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 21 |
| + | + | - | 9 |
| + | - | + | 9 |
| + | - | - | 7 |
| - | + | + | 9 |
| - | + | - | 8 |
| - | - | + | 14 |
| - | - | - | 34 |
| | Unknown | | 2 |
| | Total | | 113 |

Asthmatic reactions were accompanied by decline in $FEV_1$ to less than 50% of the baseline value in 21 periods (19%). Obstructive reactions as measured by $FEV_1$ were of 4 days duration or less in 29 cases, and longer-lasting in 17.

9-2-2-3 Seroconversions without SRI

As was mentioned in chapter 9-2-1, 39 episodes were noted in which an MRI or subclinical infection was detected by means of routinely performed serological investigations. Out of 24 MRIs, an association with exacerbation was observed in 10 instances (42%), whereas only 2 out of 15 subclinical infections (13%) coincided with exacerbation during the period in which seroconversion occurred. The exacerbations that were registered were associated with a decline in $FEV_1$ on 8 occasions, in 6 of which decreases of more than 50% were noted. Data are summarized in Table 9-5.

Table 9-5 Patterns of exacerbation in seroconversion without SRI in group I patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | MRI | Subclinical |
|---|---|---|---|---|
| + | + | + | – | 1 |
| + | + | – | 2 | – |
| + | – | + | – | – |
| + | – | – | 4 | 1 |
| – | + | + | 1 | – |
| – | + | – | – | – |
| – | – | + | 3 | – |
| – | – | – | 14 | 13 |
| | | Total | 24 | 15 |

9-2-3 Results of sputum gram stains

Seventy-one out of 158 periods of SRI (45%), experienced by group I patients, were accompanied by the production of yellow-green coloured sputum. In the course of SRI with positive serology yellow-green sputum was produced in 20 instances (44%). Fifteen samples suitable for washing and gram staining were collected. All gram stains were assessed to be positive. Subjects with SRI of unknown origin notified the production of coloured sputum 51 times (45%). Forty-three gram stains were made, 40 of which were judged positive. Nine SRIs were thought to be of primarily bacterial origin.

9-3 Group II - Asthma and chronic airflow obstruction (CAO)

9-3-1 Incidence of respiratory infection

Sixty-nine subjects were categorized into group II on the base of anamnestic and lung function data. They were supposed to have an asthmatic background like group I patients, but in almost all of them the bronchial obstructive disease had caused more or less irreversible air flow limitation. These patients took part in the study for 532 patient months, which yields an average participation of 7.7 months.

Ninety-three SRIs were experienced by these patients, which stands for

an incidence of 0.17 SRI per patient month. Positive serological results were obtained in 36 infection episodes (39%), whereas in the remaining SRIs no significant rises in antibody titre could be found. The agents involved in the SRIs with positive serology are listed in Table 9-6.

Table 9-6 Agents involved in 36 periods of SRI detected by fourfold or greater rise in antibody titre. Group II patients

| Agent | No. of SRIs with seroconversion |
|---|---|
| Influenza A virus | 24 |
| RSV | 4 |
| Parainfluenza 3 virus | 3 |
| Influenza B virus | 2 |
| Adenovirus | 2 |
| Influenza A virus + RSV | 1* |
| Total | 36 |

*double infection

Table 9-7 No. of seroconversions to various agents without the occurrence of SRI in group II patients

| Agent | No. of seroconversions | | | |
|---|---|---|---|---|
| | MRI | Subclinical infection | Clinical data unknown | Total |
| Influenza A virus | 5 | 3 | - | 8 |
| Parainfluenza 3 virus | 4 | - | - | 4 |
| Influenza B virus | 1 | 1 | - | 2 |
| RSV | 1 | 1 | - | 2 |
| *Mycoplasma pneumoniae* | - | 1 | - | 1 |
| *Chlamydia psittaci* | - | - | 1 | 1 |
| Influenza A virus + RSV | - | - | 1* | 1* |
| Total | 11 | 6 | 3 | 20 |

* double infection

Furthermore, 20 seroconversions, including a double one, were detected by means of routine serological investigations. This corresponds with 0.04 seroconversion without SRI per patient month. Eleven seroconversions could be related to MRI, whereas 6 infections passed off without any clinical symptoms. Three seroconversions, including the double one, were found, while the relevant subjects were not under observation. Clinical and serological data are summarized in Table 9-7.

9-3-2 Effects on CNSLD parameters

9-3-2-1 SRI with positive serology

Viral SRI was associated with exacerbation in 31 out of 36 periods (86%). Decrease in $FEV_1$ was registered in 20 subjects (56%), whereas increases in dyspnoea/wheezing and cough/sputum were observed in 21 (58%) and 26 (72%) instances, respectively. Various combinations of exacerbations are shown in Table 9-8.

Table 9-8 Patterns of exacerbation in the course of SRI with positive serology in group II patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 13 |
| + | + | − | 4 |
| + | − | + | 3 |
| + | − | − | − |
| − | + | + | 3 |
| − | + | − | 1 |
| − | − | + | 7 |
| − | − | − | 5 |
| | | Total | 36 |

Marked obstructive reactions, with $FEV_1$ decreasing by at least 50%, were noted in the course of 8 viral SRIs (22%) in this group. Eleven exacerbations involving $FEV_1$ lasted 4 days or shorter, whereas 9 reactions of at least 5 days were noted.

9-3-2-2 SRI without positive serology

Group II patients experienced 57 SRIs of unknown origin, 34 of which could be related to exacerbation of the bronchial obstructive disease (60%). Significant changes in $FEV_1$, dyspnoea/wheezing and cough/sputum were present in 18 (32%), 25 (44%), and 22 (39%) patients, respectively in different combinations. The patterns of exacerbation are presented in Table 9-9. Decreases in $FEV_1$ of over 50% were observed 7 times (12%). Twelve obstructive reactions were short-lasting, whereas 6 of these lasted for 5 days or more.

Table 9-9 Patterns of exacerbation in the course of SRI without positive serology in group II patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 8 |
| + | + | - | 7 |
| + | - | + | 1 |
| + | - | - | 2 |
| - | + | + | 7 |
| - | + | - | 3 |
| - | - | + | 6 |
| - | - | - | 23 |
| | Total | | 57 |

9-3-2-3 Seroconversions without SRI

Eleven periods of MRI and 6 subclinical infections were noted in group II patients. In case of MRI, 5 exacerbations (45%) were observed, 3 of these with significant changes in $FEV_1$. One subject showed an $FEV_1$ decline of over 50%. Two out of 6 subclinical infections were associated with an increase in symptoms, however, without changes in $FEV_1$. Data are summarized in Table 9-10.

Table 9-10 Patterns of exacerbation in seroconversion without SRI in group II patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | MRI | Subclinical |
|---|---|---|---|---|
| + | + | + | 1 | - |
| + | + | - | - | - |
| + | - | + | 1 | - |
| + | - | - | 1 | - |
| - | + | + | - | 1 |
| - | + | - | - | - |
| - | - | + | 2 | 1 |
| - | - | - | 6 | 4 |
| | | Total | 11 | 6 |

9-3-3 Results of sputum gram stains

Bacterial infection was suspected because of the production of yellow or green sputum in 64 out of 93 SRIs (69%). Viral symptomatic respiratory infection was attended with coloured sputum in 24 instances (67%). Nineteen suitable samples were obtained, washed, and coloured; 18 of these turned out to be positive. SRI of unknown origin was associated with the production of yellow-greenish coloured sputum in 40 of 57 episodes (70%). Thirty-four sputum gram stains were prepared and 30 of these were assessed to be positive. Eight infection periods were thought to be of bacterial origin in first instance.

9-4 Group III - Chronic airflow obstruction (CAO)

9-4-1 Incidence of respiratory infection

Of patients participating in the study, only 28 were suffering from conditions characterized by chronic dyspnoea on exertion and largely irreversible obstruction of airflow. The term pulmonary emphysema could be applied to most of them on clinical, röntgenologic and pathophysiological grounds. These subjects accounted for 204 patient months, which equals an average of 7.3 months of participation per person. Thirty-three SRIs were notified by these patients, yielding an SRI incidence

of 0.16 per patient month. A viral agent could be detected by means of serological investigation in 9 cases (27%), which are summarized in Table 9-11.

Table 9-11 Agents involved in 9 periods of SRI detected by fourfold or greater rise in antibody titre. Group III patients

| Agent | No. of SRIs with seroconversion |
|---|---|
| Influenza A virus | 6 |
| Parainfluenza 3 virus | 1 |
| RSV | 1 |
| Adenovirus | 1 |
| Total | 9 |

In addition, 6 seroconversions were detected by means of routine blood sampling, which equalizes to 0.03 seroconversion without SRI per patient month. Four significant rises in titre could be related to MRI, whereas one subclinical infection and one period with unknown data occurred. Agents involved in these episodes are listed in Table 9-12.

Table 9-12 No. of seroconversions to various agents without the occurrence of SRI in group III patients

| Agent | No. of seroconversions | | | |
|---|---|---|---|---|
| | MRI | Subclinical infection | Clinical data unknown | Total |
| Influenza A virus | 3 | | | 3 |
| RSV | 1 | 1 | 1 | 3 |
| Total | 4 | 1 | 1 | 6 |

9-4-2 Effects on CNSLD parameters

9-4-2-1 SRI with positive serology

Exacerbation was registered in 6 out of 9 viral SRIs (67%). An example is shown in case report 29 (Appendix I). Increases in dyspnoea/wheezing and cough/sputum were both noted in 5 instances (56%), whereas increased bronchial obstruction could be confirmed by means of $FEV_1$ measurements in one patient (11%) only, in whom a maximum decrease of 42% compared to baseline value was noted. Different reaction patterns are shown in Table 9-13.

Table 9-13 Patterns of exacerbation in the course of SRI with positive serology in group III patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 1 |
| + | + | - | - |
| + | - | + | - |
| + | - | - | - |
| - | + | + | 3 |
| - | + | - | 1 |
| - | - | + | 1 |
| - | - | - | 3 |
| | | Total | 9 |

9-4-2-2 SRI without positive serology

Out of 24 SRIs of unknown viral origin 15 could be related to exacerbation (63%). Dyspnoea/wheezing and cough/sputum were present on 7 (29%) and 12 (50%) occasions, respectively, whereas fall in $FEV_1$ was registered only twice (8%). One subject had a decline in $FEV_1$ of over 50%. Table 9-14 shows the different combinations of CNSLD parameters in the course of unknown SRI in these patients.

Table 9-14 Patterns of exacerbation in the course of SRI without positive serology in group III patients

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 1 |
| + | + | - | 1 |
| + | - | + | - |
| + | - | - | - |
| - | + | + | 3 |
| - | + | - | 2 |
| - | - | + | 8 |
| - | - | - | 9 |
| | | Total | 24 |

9-4-2-3 Seroconversions without SRI

None of the seroconversions, in which clinical data could be collected and SRI was absent, could be related to exacerbation.

9-4-3 Results of sputum gram stains

SRI was attended by the occurrence of yellow-greenish sputum in 23 out of 33 instances (70%). In viral SRI coloured sputum was coughed up by 7 patients (78%). Gram stains were made of 6 samples, 4 of which turned out to be positive. SRI of unknown origin coincided with yellow-greenish sputum in 16 out of 24 instances (67%). Fourteen gram stains were made, 13 of which were assessed to be positive. Five SRIs were thought to be of bacterial origin primarily.

9-5 Assessment of results

In order to investigate whether different reaction patterns exist in patients with various clinical entities within the spectrum of CNSLD, the parameters scored in the course of SRI were compared for the three groups. For every SRI of viral and unknown origin the occurrence of exacerbation, significant decrease in $FEV_1$, 50% decrease in $FEV_1$ (at least one day), increase in dyspnoea/wheezing and cough/sputum, and production

of coloured sputum were noted. Tables 9-15 and 9-16 give the results for SRI with and without positive serology, respectively. As can be seen, there seems to be a strong tendency for patients classified as asthmatics (group I) to react with increased bronchial obstruction (lowering of $FEV_1$) in viral SRI and to a lesser extent in SRI of unknown origin. Also in group II a considerable proportion of subjects show decreases in $FEV_1$. In the patients categorized in group III, however, significant changes in $FEV_1$ are rare, whereas in these patients as well as in those of group II there seems to be a greater tendency for the production of coloured sputum as compared to group I subjects.

A difficulty arises when statistical analysis has to be done on these results, as some patients experienced several SRIs, whereas others had only one. This implicates that host factors determining reaction patterns are overscored for those patients with more than one SRI. In order to avoid this problem results were reassessed and two statistical analyses were done. $\chi^2$ tests were carried out for the exacerbation parameters in the various patient groups. The statistical analyses were done, using every first and every last SRI per patient, respectively, so that only one infection per patient was taken into account in both tests. Some significant differences in the reaction patterns of the patient groups were found. Decreases in $FEV_1$ occur more frequently in patients with an asthmatic background (groups I and II) in the course of SRI ($p < 0.01$) and coloured sputum is less often observed in group I patients ($p < 0.01$). The detailed figures are summarized in Tables 9-17, 9-18, and 9-19 for viral, unknown, and all SRIs, respectively.

Table 9-15 Occurrence of changes in CNSLD parameters in SRI with positive serology according to different clinical groups

| | Total no. | With exacerbation | $FEV_1 \downarrow$ | $FEV_1 \downarrow\downarrow$ | Dyspnoea/wheezing $\uparrow$ | Cough/sputum $\uparrow$ | Yellow/green sputum |
|---|---|---|---|---|---|---|---|
| Group I | 44 | 37 | 32 | 19 | 20 | 25 | 20 |
| Group II | 36 | 31 | 20 | 8 | 21 | 26 | 24 |
| Group III | 9 | 6 | 1 | 0 | 5 | 5 | 7 |

Table 9-16 Occurrence of changes in CNSLD parameters in SRI without positive serology according to different clinical groups

| | Total no. | With exacerbation | $FEV_1 \downarrow$ | $FEV_1 \downarrow\downarrow$ | Dyspnoea/wheezing $\uparrow$ | Cough/sputum $\uparrow$ | Yellow/green sputum |
|---|---|---|---|---|---|---|---|
| Group I | 111 | 77 | 46 | 21 | 47 | 53 | 71 |
| Group II | 57 | 34 | 18 | 7 | 25 | 22 | 40 |
| Group III | 24 | 15 | 2 | 1 | 7 | 12 | 16 |

Tables 9-15 to 9-19:

Group I = asthma
Group II = asthma + chronic airflow obstruction (CAO)
Group III = CAO
$FEV_1 \downarrow$ = decrease in $FEV_1$ of $\geq$ 25% for at least 2 consecutive days
$FEV_1 \downarrow\downarrow$ = decrease in $FEV_1$ of $\geq$ 50% for at least 1 day
Dyspnoea/wheezing $\uparrow$ = increase in dyspnoea/wheezing of $\geq$ 2 points for at least 2 consecutive days
Cough/sputum $\uparrow$ = increase in cough/sputum of $\geq$ 2 points for at least 2 consecutive days

Table 9-17 Total number of first and last SRIs with positive serology and scores of different parameters of exacerbation in relation to the various clinical subgroups of CNSLD ($\chi^2$ test)

| | Total no. | With exacerbation | $FEV_1 \downarrow$ | $FEV_1 \downarrow\downarrow$ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | Yellow/green sputum |
|---|---|---|---|---|---|---|---|
| | | | First SRIs with positive serology | | | | |
| Group I | 38 | 31 | 26 | 15 | 17 | 22 | 15 |
| Group II | 28 | 24 | 16 | 7 | 16 | 20 | 18 |
| Group III | 8 | 5 | 1 | 0 | 5 | 4 | 6 |
| | | NS | $p < 0.02$ | $p<0.10$ | NS | NS | $p< 0.10$ |
| | | | Last SRIs with positive serology | | | | |
| Group I | 38 | 33 | 27 | 15 | 18 | 23 | 16 |
| Group II | 28 | 23 | 15 | 6 | 17 | 18 | 18 |
| Group III | 8 | 5 | 1 | 0 | 4 | 4 | 6 |
| | | NS | $p<0.01$ | $p<0.05$ | NS | NS | $p<0.10$ |

Table 9-18 Total number of first and last SRIs without positive serology and scores of different parameters of exacerbation in relation to the various clinical subgroups of CNSLD ($\chi^2$ test)

| | Total no. | With exacerbation | $FEV_1\downarrow$ | $FEV_1\downarrow\downarrow$ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | Yellow/green sputum |
|---|---|---|---|---|---|---|---|
| **First SRIs without positive serology** | | | | | | | |
| Group I | 71 | 49 | 32 | 11 | 27 | 33 | 30 |
| Group II | 40 | 26 | 14 | 5 | 19 | 17 | 27 |
| Group III | 17 | 9 | 2 | 0 | 5 | 6 | 10 |
| | | NS | p<0.05 | NS | NS | NS | p<0.05 |
| **Last SRIs without positive serology** | | | | | | | |
| Group I | 71 | 45 | 29 | 14 | 27 | 34 | 31 |
| Group II | 40 | 25 | 13 | 5 | 19 | 18 | 30 |
| Group III | 17 | 10 | 2 | 0 | 5 | 8 | 10 |
| | | NS | p<0.10 | NS | NS | NS | p<0.01 |

Table 9-19 Total number of all first and last SRIs and scores of different parameters of exacerbation in relation to the various clinical subgroups of CNSLD ($\chi^2$ test)

| | Total no. | With exacerbation | $FEV_1\downarrow$ | $FEV_1\downarrow\downarrow$ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | Yellow/green sputum |
|---|---|---|---|---|---|---|---|
| | | | First SRIs | | | | |
| Group I | 109 | 80 | 58 | 26 | 44 | 55 | 45 |
| Group II | 68 | 50 | 30 | 12 | 35 | 37 | 45 |
| Group III | 25 | 14 | 3 | 0 | 10 | 10 | 16 |
| | | NS | p<0.01 | p<0.05 | NS | NS | p<0.01 |
| | | | Last SRIs | | | | |
| Group I | 109 | 78 | 56 | 29 | 45 | 57 | 47 |
| Group II | 68 | 48 | 28 | 11 | 36 | 36 | 48 |
| Group III | 25 | 15 | 3 | 0 | 9 | 12 | 16 |
| | | NS | p<0.01 | p<0.01 | NS | NS | p<0.01 |

# CHAPTER 10
# RESULTS RELATED TO SEROCONVERSION TO DIFFERENT INFECTING AGENTS

## 10-1 Introduction

For a comparison of effects brought about by the various agents tested for in the study, the results were readjusted according to seroconversion to the different viruses, *Mycoplasma pneumoniae*, and *Chlamydia psittaci*. Clinical diagnoses regarding CNSLD subgroups were not taken into account, as in all groups the distribution of various agents was more or less similar, influenza A accounting for about 2/3 of all SRIs with positive serology, and for about 2/5 of all seroconversions without SRI. Seroconversions were reexamined and the occurrence of possible differences with regard to clinical picture, effects on CNSLD parameters, and bacterial superinfection were investigated for the various infecting agents. In this chapter, the results of sputum gram stains will be presented together with the effects on CNSLD parameters.

## 10-2 Influenza A virus

### 10-2-1 Epidemiology and clinical picture

In the course of the study, 94 fourfold or greater rises in titre to influenza A virus were found. Of these seroconversions 63 (67%) were related to SRI, according to the criteria given in chapter 7-3-1, whereas 19 (20%) could be associated with MRI and 10 (11%) remained clinically undetected. In 2 instances (2%) clinical data were unknown. Epidemics due to influenza A infection were observed in all living-units with outbreaks in March 1977 and December 1977 in group A, in February 1976 and March 1977 in group B, and in February 1976, March 1977, and January 1978 in group C. Epidemics did not occur during the winter of 1978/1979, but the rate of influenza vaccination was higher than in the previous seasons then.

Of the seroconversions with known clinical data, 76 (83%) occurred in the months of December, January, February, and March; 55 of the episodes

were associated with SRI, whereas 18 MRIs were noted and only 3 infections remained subclinical. On the other hand, 16 rises in titre (17%) were found outside the influenza seasons, 7 of which were subclinical, whereas 8 were related to SRI and 1 to MRI.

10-2-2 Effects on CNSLD parameters

10-2-2-1 Influenza A virus with SRI

Sixty-three SRIs due to infection by influenza A virus were observed. In the course of these episodes a total of 53 (84%) exacerbations were noted. Augmented dyspnoea/wheezing was observed in 33 (52%) instances, and increased productive coughing in 37 (59%), whereas significant changes in $FEV_1$ were noted on 43 occasions (68%). Results are summarized in Table 10-1.

Table 10-1 Patterns of exacerbation in influenza A infection, associated with SRI

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 18 |
| + | + | - | 11 |
| + | - | + | 10 |
| + | - | - | 4 |
| - | + | + | 3 |
| - | + | - | 1 |
| - | - | + | 6 |
| - | - | - | 10 |
| | | Total | 63 |

$FEV_1$ decreased by more than 50% of the baseline value in 22 subjects (35%). Thirty-six patients (57%) stated to produce yellow-green sputum during the episode of infection. Twenty-seven sputum samples suitable for staining were handed in, gram preparations were positive in 25 of these.

10-2-2-2 Influenza A virus without SRI

Seroconversion to influenza A virus without SRI was associated with exacerbation in 6 out of 29 instances (21%). Four MRIs and 2 subclinical infections were related to exacerbation, which was on all occasions accompanied by decreases in $FEV_1$. Dyspnoea/wheezing as well as cough/sputum increased in 2 subjects. Data are shown in Table 10-2.

Table 10-2 Patterns of exacerbation in influenza A infection, not associated with SRI

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | MRI | Subclinical |
|---|---|---|---|---|
| + | + | + | – | 1 |
| + | + | – | 1 | – |
| + | – | + | 1 | – |
| + | – | – | 2 | 1 |
| – | + | + | – | – |
| – | + | – | – | – |
| – | – | + | – | – |
| – | – | – | 15 | 8 |
| | | Total | 19 | 10 |

10-3 Parainfluenza viruses

10-3-1 Epidemiology and clinical picture

Seroconversions to parainfluenza 3 virus were detected in 23 paired serum samples and to parainfluenza 1 virus once. This last infection and 8 of the parainfluenza 3 infections were related to SRI, whereas 10 MRIs, 1 subclinical infection and 4 episodes in which patients were not under observation were noted. Parainfluenza infections were scattered throughout the study period with relatively few seroconversions in winter. In the months of December to March only 4 rises in titre were found. Epidemic outbreaks did not occur.

10-3-2 Effects on CNSLD parameters

10-3-2-1 Parainfluenza viruses with SRI

Out of 9 SRIs caused by parainfluenza viruses, 6 were associated with exacerbation. SRI due to parainfluenza 1 infection did not show signs of exacerbation. Productive coughing was observed on 5 occasions and increases in dyspnoea/wheezing on 4, whereas $FEV_1$ decreased in 3 subjects, 2 of these showing values below 50% of the baseline value.

The obstructive changes observed lasted 5 days in one patient and 10 days in the remaining 2 subjects. The patterns of exacerbation are shown in Table 10-3. Four patients notified production of yellow-green sputum; 3 gram stains were made, all of which turned out to be positive.

Table 10-3 Patterns of exacerbation in infection by parainfluenza viruses associated with SRI

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 2 |
| + | + | - | 1 |
| + | - | + | - |
| + | - | - | - |
| - | + | + | 1 |
| - | + | - | - |
| - | - | + | 2 |
| - | - | - | 3 |
| | | Total | 9 |

10-3-2-2 Parainfluenzaviruses without SRI

Ten seroconversions to parainfluenza 3 virus could be associated with MRI. In 6 instances a concomitant exacerbation was observed. Productive coughing was most frequent, namely in 5 patients, whereas decreasing $FEV_1$ and increasing dyspnoea/wheezing occurred twice and once, respectively. Subclinical parainfluenza 3 infection was not associated with exacerbation. Data are summarized in Table 10-4. Of 4 infection periods with unknown clinical data, 1 anamnestic exacerbation was reported,

whereas in the remaining 3 this was not so.

Table 10-4 Patterns of exacerbation in infection by parainfluenza viruses not associated with SRI

| $FEV_1\downarrow$ | Dyspnoea/wheezing↑ | Cough/sputum↑ | MRI | Subclinical |
|---|---|---|---|---|
| + | + | + | 1 | - |
| + | + | - | - | - |
| + | - | + | - | - |
| + | - | - | 1 | - |
| - | + | + | - | - |
| - | + | - | - | - |
| - | - | + | 4 | - |
| - | - | - | 4 | 1 |
| | | Total | 10 | 1 |

10-4 Respiratory syncytial virus (RSV)

10-4-1 Epidemiology and clinical picture

A total of 19 single seroconversions to RSV were found. In group A 2 outbreaks were observed in February, 1977 and February-March, 1979, which accounted for 11 infections. Sixteen of the seroconversions were found in the winter seasons.

RSV was associated with signs of SRI in 6 patients, whereas 7 MRIs and 4 subclinical infections were registered, and clinical data were insufficiently collected in 2 subjects.

10-4-2 Effects on CNSLD parameters

10-4-2-1 RSV with SRI

All seroconversions to RSV associated with SRI could be related to exacerbation. Increasing cough/sputum scores were obtained in all cases, whereas dyspnoea/wheezing increased in 3 subjects, and declining $FEV_1$ was observed once. Data are shown in detail in Table 10-5. Four patients coughed up coloured sputum and 3 of them handed in suitable samples, of which gram stains came out positively.

Table 10-5 Patterns of exacerbation in SRI by respiratory syncytial virus

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 1 |
| + | + | - | - |
| + | - | + | - |
| + | - | - | - |
| - | + | + | 2 |
| - | + | - | - |
| - | - | + | 3 |
| - | - | - | - |
| | | Total | 6 |

10-4-2-2 RSV without SRI

Four out of 7 MRIs and 1 out of 4 subclinical infections were related to exacerbation. Four periods were accompanied by augmented cough/sputum, 3 by increased dyspnoea/wheezing, and 2 by declining $FEV_1$. In 3 MRIs yellow/green sputum was coughed up. Two gram stains were made, both being positive. Data are summarized in Table 10-6. In both infections

Table 10-6 Patterns of exacerbation in infection by respiratory syncytial virus, not associated with SRI

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | MRI | Subclinical |
|---|---|---|---|---|
| + | + | + | 1 | - |
| + | + | - | - | - |
| + | - | + | - | - |
| + | - | - | 1 | - |
| - | + | + | 1 | 1 |
| - | + | - | - | - |
| - | - | + | 1 | - |
| - | - | - | 3 | 3 |
| | | Total | 7 | 4 |

in which clinical data were not satisfactorily collected exacerbation was supposed to have occurred retrospectively.

10-5 Influenza B virus

10-5-1 Epidemiology and clinical picture

Seroconversion to influenza B virus was found 13 times. Concomitant SRI was observed in 7 patients. Six of the seroconversions with SRI were detected in the winter months and one in April. Two MRIs were registered, one in winter and one in autumn, whereas 4 subclinical infections all occurred out of the winter season. Outbreaks as seen by influenza A infections were not observed.

10-5-2 Effects on CNSLD parameters

10-5-2-1 Influenza B virus with SRI

In one patient data were not collected properly and conclusions could not be drawn. In the remaining 6 subjects with SRI due to influenza B virus, 4 exacerbations were noted with $FEV_1$ declining in 3, and productive coughing and dyspnoea/wheezing increasing in 3 and 2 subjects, respectively. Table 10-7 shows the various patterns. Four patients

Table 10-7 Patterns of exacerbation in influenza B infection, associated with SRI

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | |
|---|---|---|---|
| + | + | + | 1 |
| + | + | - | - |
| + | - | + | 1 |
| + | - | - | 1 |
| - | + | + | 1 |
| - | + | - | - |
| - | - | + | - |
| - | - | - | 2 |
| | Unknown | | 1 |
| | Total | | 7 |

coughed up coloured sputum and handed in suitable specimens. Three gram stains were judged to be positive, whereas in the remaining preparation no bacteria could be found.

10-5-2-2 Influenza B virus without SRI

None of the patients with MRI or subclinical influenza B infection showed signs of exacerbation of CNSLD.

10-6 Remaining single seroconversions

10-6-1 Adenovirus

Significant rises in titre to the antigen of the group of adenoviruses were found in 5 patients. Three SRIs and 1 MRI were related to exacerbation, whereas a subclinical infection was not. Two bacterial superinfections were noted.

10-6-2 *Mycoplasma pneumoniae*

Evidence of *Mycoplasma pneumoniae* infection was detected serologically in 2 patients only. One SRI was associated with a severe asthmatic episode in a 13-year-old boy (see case report 21, Appendix I), whereas in another patient no symptoms of upper respiratory tract infection were

Table 10-8 Patterns of exacerbation in infection by adenovirus and *Mycoplasma pneumoniae*

| $FEV_1$ ↓ | Dyspnoea/wheezing ↑ | Cough/sputum ↑ | SRI | MRI | Subclinical |
|---|---|---|---|---|---|
| + | + | + | Ad,Mp* | – | – |
| + | + | – | – | – | – |
| + | – | + | – | – | – |
| + | – | – | – | Ad | – |
| – | + | + | Ad | – | – |
| – | + | – | Ad | – | – |
| – | – | + | – | – | Mp |
| – | – | – | – | – | Ad |

*Ad = adenovirus; Mp = *Mycoplasma pneumoniae*

found, but productive coughing increased markedly. The data for adenovirus and *Mycoplasma pneumoniae* are shown together in Table 10-8.

10-6-3 *Chlamydia psittaci*

In one patient seroconversion to *Chlamydia psittaci* was detected during the school holidays, which he spent with his parents in Davos. Although clinical data were not registered sufficiently, it could be found that no symptoms of upper respiratory tract infection were observed in the relevant period. However, a distinct asthmatic exacerbation was reported, which could be objectivied by some lung function measurements in which $FEV_1$ was below 50% of the normal baseline value for this patient.

10-7 Double seroconversions

Five double infections were registered in the course of the study. In 3 of these episodes clinical data were not fully known, because the relevant subjects were not under observation at that time. One patient had an SRI with decreasing $FEV_1$ as well as augmentation of dyspnoea/wheezing and cough/sputum. Moreover, he experienced a bacterial superinfection. One subclinical infection without signs of exacerbation was noted. In 2 of the out-clinic patients some anamnestic data regarding SRI and exacerbation could be registered. Results regarding all double seroconversions are listed in Table 10-9.

Table 10-9 Occurrence of SRI and exacerbation in infection by more than one agent

| Serological findings | SRI | Exacerbation | Remarks |
|---|---|---|---|
| Influenza A virus/RSV | + | + | |
| Influenza A virus/RSV | + | + | Anamnestic data only |
| *M.pneum.*/parainfl.3 virus | + | + | Ibid |
| RSV/ parainfluenza 3 virus | - | - | |
| RSV/*Chlamydia psittaci* | ? | ? | |

10-8 Summary of results

When the above-mentioned results are combined, including only those seroconversions in which clinical data and exacerbation parameters could be collected well enough to draw conclusions, it appears that a great deal of all seroconversions that were found are associated with signs of exacerbation of CNSLD. In general, SRI is related to exacerbation more often than MRI and subclinical infection, as was shown in chapter 8-5. This seems to be true for all infecting agents, but only to a small extent for parainfluenza 3 virus and RSV, in which a considerable proportion of MRI was concomitant with exacerbation. Influenza B was less often related to exacerbation than the other offending agents, but figures are too small to draw any definite conclusions. The various infecting agents as found by means of significant rises in titre in the course of the study period, and the extent to which they were linked to signs of SRI and exacerbation are listed in Table 10-10.

Table 10-10 Seroconversions to different infectious agents and their relation to clinical picture and exacerbation

| Agent | No.of seroconversions with known clinical data | No.with exacerbation (%) | No.of SRIs/ no.with exacerbation | No.of MRIs/ no with exacerbation | No.of subclinical infections/ no.with exacerbation |
|---|---|---|---|---|---|
| Infl.A | 92 | 59(64%) | 63/53 | 19/ 4 | 10/ 2 |
| Parainfl. | 20 | 12(60%) | 9/ 6 | 10/ 6 | 1/ 0 |
| RSV | 17 | 11(65%) | 6/ 6 | 7/ 4 | 4/ 1 |
| Infl.B | 12 | 4(33%) | 6/ 4 | 2/ 0 | 4/ 0 |
| Adeno | 5 | 4(80%) | 3/ 3 | 1/ 1 | 1/ 0 |
| *M.pneum.* | 2 | 2(100%) | 1/ 1 | 0/ 0 | 1/ 1 |
| Total | 148 | 92(62%) | 88/73 (83%) | 39/15 (38%) | 21/ 4 (19%) |

# CHAPTER 11
# DISCUSSION OF RESULTS

## 11-1 Incidence of respiratory infection

### 11-1-1 Introduction

In the present study symptomatic respiratory infection (SRI) was supposed to be present if 2 or more of the symptoms, mentioned in chapter 7-3-1, were present for at least 36 hours. This threshold was chosen in order to avoid a high number of false positive episodes. False positive SRIs may occur in case of allergic rhinitis with symptoms like rhinorrhoea and sneezing and therefore special attention was given to the occurrence of these episodes. Combinations of these symptoms alone were few and almost always associated with systemic effects like malaise and fever, so that it can be assumed that allergic rhinitis did not give rise to a considerable number of false positive SRIs. Comparison of SRI frequencies in patients with CNSLD to those in other reports is difficult for various reasons:

- Many studies on the relationship between viral respiratory infection and flare-ups of CNSLD use the occurrence of an exacerbation of asthmatic or bronchitic symptoms as the moment from which on virological studies are started. The number of SRIs that take place without exacerbation is usually not mentioned.
- The definition of SRI is not identical in different studies. In most reports patients were asked to notify any acute respiratory illness, but the threshold to call any episode as such is usually not set.
- Compositions of study groups, for example with regard to age, differ strongly in various studies. Likewise, observation periods vary from one report to another.

In spite of these difficulties, some data can be compared to the results presented in this thesis.

11-1-2 SRI in asthma

Data with regard to the frequency of respiratory infection in patients categorized as asthmatics are scarce and almost exclusively limited to children. McIntosh *et al.*[170] found a high frequency of respiratory infection of proven viral origin in young asthmatic children, namely 0.43 per patient month. Their study was limited to 2 subsequent winter seasons, whereas no information was collected during summer periods. Upper respiratory infection was defined as either rhinorrhoea or pharyngitis, or both, and otitis media was considered separately. In a group of 41 children, aged 13-17 years, and 8 adults, aged 22-60 years, Minor *et al.*[183] found 128 episodes of SRI, employing a threshold of one symptom of upper respiratory tract infection in the course of 392 patient months, which is equal to 0.33 SRI per patient month. In another study by these authors[184] of 16 children aged 3-11 years, incidences of 0.6 SRI (threshold: one symptom of upper respiratory tract infection) per patient month and 0.38 severe SRI (threshold: 2 symptoms and/or fever) were found.

11-1-3 SRI in chronic bronchitis

There are some studies from which SRI rates in patients diagnosed as "chronic bronchitics" can be calculated. Stenhouse[250] found 38 upper respiratory tract infections in 255 patient months, which equals a frequency of 0.15, whereas Eadie *et al.*[64] observed 75 episodes in 435 patient months, which stands for an incidence of 0.17. These figures correspond well with the group III patients in this study, in which a rate of 0.16 SRI per patient month was observed.

11-1-4 SRI with positive serology

In the survey 90 out of 284 episodes of SRI (31.7%) could be related to viral infection by means of systematically conducted serological investigations. This percentage is comparable to those found by others in similar studies, although in most of these studies isolation techniques were used in combination with serology. Table 11-1 summarizes the results of some previous reports.

Table II-1 Frequencies of virus detection in the course of SRI in different studies

| Author(s) | No. of SRIs | No. of SRIs of proven viral origin | Percentage |
|---|---|---|---|
| Eadie *et al.*[64] | 75 | 16 | 21 |
| Minor *et al* [183] | 128 | 33 | 26 |
| Löwenberg & Orie[166] | 282 | 87 | 31 |
| Horn *et al.*[115] | 1934 | 614 | 32 |
| Gregg[95] | 2635 | 891 | 34 |
| Lambert & Stern[148] | 73 | 29 | 40 |
| Minor *et al.*[184] | 78 | 38 | 49 |
| Present study | 284 | 90 | 32 |

Thoroughly conducted serological investigations offer a good possibility to detect a substantial part of demonstrable viral infections, and the use of isolation techniques does not add much information if both methods are combined. Gump *et al.*[101] compared both techniques and found that 81% of the viral infections they were able to demonstrate, were detected by serology alone, whereas 14% were detected by isolation alone.

However, the use of serology alone has one major disadvantage, namely the impossibility to measure infection rates with common cold viruses, which are divided morphologically into the rhinoviruses and coronaviruses. There is a great multiplicity of different types of these agents with relative immunological specificity. At present over 100 different antigenic rhinovirus types are known. Therefore, cell culture is the standard method for rhinovirus isolation, and propagation and serological study is possible only when the types of circulating viruses are known from viral cultures. As these agents tolerate the process of freezing and thawing less well than other viruses, in the present survey it was impossible to collect reliable information with regard to infection rates with common cold viruses.

11-1-5 Comment

The incidences of respiratory infection in asthmatics found in studies mentioned in chapter 10-1-2 are higher than the present ones, even if SRI and MRI are combined, in which case a frequency of 0.23 episode per patient month can be estimated. However, reports describe results in younger children. These data are in agreement with results of large population surveys. The incidence of acute respiratory tract infection is highest in infants under 1 year of age with a second peak in children aged 5 years. There is a sharp decrease in rates to the age of 10 years, after which a more or less equal frequency for the different age groups can be found as was shown by Miller in a study by the Public Health Laboratory Service and the Medical Research Council's Committee on Acute Respiratory Virus Infections.[181]

In the survey described in this thesis SRI rates did not differ significantly for the various age groups A, B, and C, although the group of youngsters showed a somewhat lower incidence. This could be due to the fact that children tended to deny any respiratory symptoms for fear of regular vena punctures because of SRI. In this respect it is interesting that the frequency of seroconversion related to MRI and subclinical infection was highest in this group: 0.05 per patient month compared to 0.03 for both other groups.

Different degrees of irreversible functional disability within the spectrum of CNSLD do not seem to be associated with varying rates of symptomatic respiratory infection, as similar frequencies were found in groups I, II, and III: 0.15, 0.17, and 0.16 SRI per patient month, respectively.

The impossibility to detect infections by common cold viruses is a deficiency of importance in the present study. Rhinoviruses have been shown to be important agents in producing exacerbations in asthmatics [95] [183] [184] [185] as well as in chronic bronchitics.[64] [172] [250] There are only few data concerning coronaviruses in this respect which indicate that these agents are also able to induce flare-ups in asthma[170] and chronic bronchitis.[101]

11-2 Exacerbations of CNSLD in the course of respiratory infection

11-2-1 Introduction

One of the major problems in comparing results of the present study to those found by other investigators is the absence of a universally accepted definition of exacerbation. In CNSLD the symptoms fluctuate from day to day, so that for the patient it is difficult to always recognize the onset of a worsening of symptoms. Furthermore, different definitions of exacerbation are usually applied to the various symptom complexes within the spectrum of CNSLD. As patients suffering from conditions covered by the omnibus term CNSLD are characterized by the presence of cough, sputum, and breathlessness with wheezing, it seems logical to consider all these symptoms when rating exacerbations. In other words, information is incomplete if exacerbations in asthma are only characterized by increased breathlessness with wheezing, and by change in production and colour of sputum in chronic bronchitis, as is usually done. In the present study it was possible to combine subjective data of symptom scores with more objective daily $FEV_1$ registrations, which adds substantial information and allows the follow-up of bronchial obstructive changes in respiratory infection. In defining the term exacerbation a threshold of 2 days persistance of increased symptoms and/or decreased $FEV_1$ was chosen in order to avoid the problem of calling one bad day an exacerbation. In literature no attention is given to the problem of relating increased symptoms to a certain time limit, which makes comparison to other studies even more difficult. Of course, the threshold set for exacerbation in the present study is arbitrarily chosen as well, but at least the term has the advantage of being well-defined (see chapter 7-3-2).

11-2-2 Exacerbation in infections of different clinical severity

Exacerbation rates were associated with the severity of clinical symptoms caused by viral infection. Out of 150 episodes with positive serology in which parameters were satisfactorily collected, 89 were related to SRI, 39 to MRI, and 22 to subclinical infection. In SRI 74 exacerbations (83%) were notified, whereas MRI was related to exacerbation in 15 (38%) and subclinical infection in only 4 (18%) instances.

11-2-3 Patterns of exacerbation in different clinical states of CNSLD

11-2-3-1 Exacerbation in asthma

As was described in chapter 9, a total of 44 SRIs with positive serology and known clinical data were found in a group of 128 patients classified as asthmatics according to criteria mentioned in chapter 7. Exacerbations were observed in 37 patients (84%). If dyspnoea and wheezing would have been the only parameters under investigation, an exacerbation rate of 45% would have been found. Increases in productive coughing were noted in 25 instances, equal to 57%. Distinct changes in $FEV_1$, however, were registered in a majority of patients: 32 (73%) scored 25% or less of the baseline value during at least 2 consecutive days. This means that for these patients $FEV_1$ was a more sensitive parameter than subjective symptom scores, which is an important finding if the results are to be compared to other studies.

Minor *et al.*[183 184] found 24 periods of asthma in the course of 38 viral SRIs (63%) in children and 17 wheezy episodes in 32 viral infections (53%) in a group of 49 persons aged 3-60 years. In both studies, patients with allergic asthma were excluded and only subjects with 4 or more attacks of asthma associated with respiratory illness during the previous year were selected. Severity of asthma was rated on a 5 points scale, and asthma was considered to be associated with symptomatic respiratory infection if it occurred any time during the first 3 days of illness and if it was greater than score 1 in severity. Huhti *et al.*[121] found somewhat higher exacerbation rates. Out of 43 SRIs of proven viral origin 33 (77%) coincided with a worsening of asthma as scored in a symptom book. Furthermore, 6 out of 16 infections (38%) noted by seroconversion in routinely collected blood samples were associated with asthma. This figure is slightly higher than in the present group, in which 19 out of 61 MRIs and subclinical infections (31%) could be related to exacerbation. Huhti *et al.*[121] did not find any indication of differences with regard to the type of asthma being intrinsic or extrinsic and whether or not the patients were treated with corticosteroids. This absence of relation was also noted when the data in the present study were examined in this respect.

The episodes of SRI without positive serology were associated with

exacerbation in 77 out of 113 instances (68%) in the asthma group. The various parameters showed changes compatible with exacerbation in about equal frequencies: dyspnoea and wheezing increased in 47 subjects, productive coughing in 53, and $FEV_1$ decreased in 46 patients. It can be assumed that besides infections undetectable by presently available diagnostic procedures and infections by still unknown agents, common cold viruses were responsible for a substantial part of these episodes.

In this respect, it is interesting to notice that the percentage of exacerbations is identical to the figures found in rhinovirus infections. Minor *et al.* in studies already mentioned[183 184] observed 24 exacerbations in 39 rhinoviral SRIs (62%), whereas Gregg[95] reported 62 wheezy episodes in the course of 93 rhinovirus infections (67%). Of the pharyngitis episodes caused by group A streptococci, none resulted in asthmatic reactions. This finding confirms clinical observations by Freeman.[79]

11-2-3-2 Exacerbation in chronic airflow obstruction (CAO)

Exacerbation rates in SRI with and without positive serology were almost identical in group III patients, namely 67% and 63%, respectively. These figures correspond with those found by other authors in chronic bronchitis. Percentages of exacerbation vary from 50 to 82 in different reports. In almost all studies increase in productive coughing has been used as the sole parameter of exacerbation. Also in the present study this symptom was observed most frequently, namely in 56% of viral and in 50% of unknown SRIs.

Nevertheless, dyspnoea and wheezing seem to be factors which should be given attention as well, as they increased to 36% of all SRIs in this group, almost as high as the 42% found in the asthma group. However, $FEV_1$ changed significantly less often in the patients with distinct irreversible bronchial obstructive syndromes than it did in asthmatics. On the other hand, SRI was more frequently associated with the production of coloured sputum in CAO than it was in asthmatic patients.

11-2-3-3 Exacerbation in asthma and chronic airflow obstruction (CAO)

Exacerbation rates in asthmatic patients with partly irreversible

changes were very similar to group I subjects: 86% for viral and 60% for unknown SRI. These figures can hardly be compared to data from the literature, because all studies are limited to patients classified as either asthmatics or chronic bronchitics. However, patterns of exacerbation can be related to those of group I and group III patients. In this respect, it is interesting to note that group II patients for a great deal tend to react asthmatic, because changes in $FEV_1$ are rather high: 56% in case of viral and 32% in unknown infections. On the other hand, there is a tendency toward features of group III, because like in the CAO group the production of coloured sputum during SRI occurs more frequently than in the asthma group.

11-2-4 Comment

Differences in exacerbation rates related to variations in clinical severity of respiratory tract symptoms have been observed before. In the study by Minor *et al.*[184] subclinical infections failed to exacerbate asthma totally, and MRIs were related to wheezing episodes in 2 out of 12 instances only, whereas SRIs caused flare-ups in 21 out of 23 episodes.

Although most subclinical infections passed off without increasing CNSLD symptoms in the present study, some distinct bronchial obstructive episodes were noted. Some increases in obstructive signs and symptoms were also observed following influenza vaccination. These observations possibly have important theoretical implications, which will be discussed in the next chapter.

The exacerbation rates associated with viral SRI in the groups with distinct asthmatic symptoms are the highest reported hitherto, whereas the figures for patients with chronic airflow obstruction are in agreement with those reported for chronic bronchitis. This is probably due to the attention given to more than one parameter in rating exacerbation. Especially in groups I and II, $FEV_1$ turned out to be a valuable parameter, and decreases in $FEV_1$ accounted for a great deal of flare-ups. On the other hand, in group III patients significant changes in $FEV_1$ were rare, whereas increasing productive coughing was responsible for a majority of exacerbations. On account of differences in patterns

of reaction between patients with reversible and largely irreversible airflow obstruction, it could be suggested that these clinical entities have different pathogenetic bases and are not as closely related as supposed by those who support the concept of one fundamental background for CNSLD. However, the figures found in a group of patients with asthmatic attacks in the past or at present, and signs of partly irreversible changes, rather suggest that there is a state of transition between both clinical entities. Many clinicians would probably call this symptom complex asthmatic bronchitis, indicating the presence of asthmatic and bronchitic symptoms. In the present study patients classified into this group indeed showed characteristics of both asthma and chronic airflow obstruction in the course of an SRI, be it of proven viral origin or not. Accordingly, it seems appropriate to consider clinical syndromes associated with generalized bronchial obstruction as a continuum with at one extreme patients with complete reversibility of obstruction reacting on SRI mainly with asthmatic symptoms (dyspnoea/wheezing ↑, $FEV_1$ ↓↓, but also cough/sputum ↑), and on the other pole patients with largely irreversible obstruction, in whom SRI tends to show mainly bronchitic signs (cough/sputum ↑↑, dyspnoea/wheezing ↑, however, hardly $FEV_1$ ↓).

Pathogenetic mechanisms, to be discussed in chapter 12, are likely to be important in CNSLD in general, but seem to predominate to various extents in different clinical entities of the disease.

## 11-3 Bacteriological investigations

### 11-3-1 Results

Gram stain preparations of carefully washed sputum samples were made in 40 out of 51 viral SRIs in which yellow-greenish sputum was expectorated. Of all 90 episodes of viral SRI a positive gram stain was found in 37, which is equal to 41%. Of 194 SRIs without positive serology, 5 were due to streptococcal pharyngitis. Of the remaining 189 episodes, 107 were associated with the production of coloured sputum. Ninety-one gram stains were made and 83 of these were considered to be positive. Hence a positive gram stain was found in 44% of episodes of SRI without positive serology. If the assumption is made that positive gram stains

would have been found in equal frequencies in those instances in which coloured sputum was expectorated but no samples were handed in, it can be estimated that bacterial sputum infection can be detected by means of gram staining in 52% of episodes of SRI with or without positive serology.

Group I patients, with the above assumption made, in the course of 45 viral infections would have bacterial superinfection in 20 instances (44%), whereas in group II + III this would be the case in 27 out of 45 viral SRIs (60%). This last figure is in accordance with data given by Smith *et al.*[242] who, using bacterial culture techniques, were able to show 102 bacterial infections in the course of 168 SRIs of viral origin (61%) in patients diagnosed as chronic bronchitics. Löwenberg and Orie[166] also presented comparable figures, as they found 7 bacterial sputum infections in the course of 13 viral SRIs (54%) in nurses suffering from CNSLD. These authors consider virus-induced increase in bronchial obstruction as an important local cause of secondary bacterial infection.[207] In reviewing the present data expectoration of yellow-green sputum with positive gram stain was preceeded by increased bronchial obstruction in approximately 50% of instances. Obstructive changes indeed seem to be of importance as a predisposing moment with regard to bacterial infection. However, it is not an obligatory factor in this respect.

II-3-2 Comment

The most important aim of the present study was to investigate to which extent respiratory viral and mycoplasmal infections cause exacerbations in patients with different clinical syndromes belonging to the group of CNSLD. Several reports suggest that there is no association between bacterial respiratoy tract infection and asthma[13 70 148 170 184 235] and also in patients with chronic bronchitis, bacteria do not seem to play a major role in causing exacerbations.[25 54 101] In view of these observations and because bacteriological laboratory facilities were poor, especially at the beginning of the study, it was decided not to give extensive attention to bacteriological investigations. Sputum had to be sent to other laboratories for culturing and for economical reasons it was

impossible to do this routinely in the course of every SRI in which sputum was expectorated. On the other hand, it has been shown that viral-bacterial interrelationships are of importance in patients with CNSLD, and Stuart-Harris[256] even suggested that the combination of viral and bacterial infection accounts for the chronic smouldering phases of the disease.

Therefore, it was decided to do small scale bacteriological studies and to accept the presence of bacteria in sputum gram stains, washed according to the method of Mulder[195], as evidence of bacterial sputum infection. Although this method of investigation does not offer complete information, as bacteria should be identified by culture methods, it is not incorrect, provided that the sputum sample is well selected and properly treated, and the technique is still in use nowadays.[179]

The results of gram staining are presented throughout the chapters in which results are described, but for the statistical analysis given in chapter 10, expectoration of yellow-green sputum was used, as this parameter was scored daily for every subject, whereas sputum samples suitable for preparation and staining were available in a proportion of cases only.

In the SRIs presented as streptococcal pharyngitis, β haemolytic group A streptococci were cultured from pus taken by pharyngeal swabs, so that in these subjects bacteriological diagnosis seems to be sufficiently established.

11-4 Exacerbation related to infecting agent

11-4-1 Results

Data presented in chapter 10 show that all of the agents to which significant rises in antibody titre were found are capable of provoking flare-ups of CNSLD symptoms. Infection by all viruses except influenza B gave rise to exacerbation in a majority of instances, even if SRI was combined with MRI and subclinical infection. Highest rates were found for *Mycoplasma pneumoniae* and the adenovirus group, but seroconversions to these agents were too few to set value on this observation.

Almost identicial exacerbation rates, varying from 60% to 70%, were found in RSV, influenza A, and parainfluenza infections. Influenza B

was related to exacerbation in 4 out of 12 infections only. MRI and subclinical infection were never associated with exacerbation in influenza B, whereas all other viruses and *Mycoplasma pneumoniae* provoked flare-ups at least once in seroconversions with absent or mild clinical symptoms.

11-4-2 Comment

It is difficult to compare results observed in infections by different viruses to those found in other studies. The absence of uniformity in patient groups with regard to age and diagnosis as well as differences in defining SRI and exacerbation, and in laboratory methods are factors that should be taken into account if comparisons are made. Nevertheless, it is interesting to summarize the results of some reports in which exacerbation rates for the various agents are mentioned. This is done in Table 11-2. In general, exacerbation rates for different agents in

Table 11-2 Number of infections with exacerbation related to number of all infections observed for different viral agents and *Mycoplasma pneumoniae*. Results of various reports

| | Infl.A | Infl.B | Parainfl. 1,2,3 | RSV | Adeno | *M.pneum.* |
|---|---|---|---|---|---|---|
| Minor[184] | 4/ 5* | - | 1/ 2 | 2/ 3 | 2/ 3 | - |
| Minor[183] | 6/ 6 | 0/ 1 | 0/ 3 | - | 0/ 4 | 0/ 1 |
| Stark[249] | - | 9/ 13 | 4/ 10 | - | - | - |
| McNamara[172] | - | - | - | 5/ 6 | - | 4/ 5 |
| McIntosh[170] | 0/ 8 | 1/ 3 | 12/ 27 | 19/ 20 | 4/ 13 | - |
| Gump[101] | 14/ 23 | 1/ 3 | 9/ 18 | 5/ 7 | 3/ 3 | - |
| Lamy[149] | 7/ 9 | 7/ 9 | 12/ 15 | 3/ 7 | 0/ 3 | 1/ 2 |
| Present study | 59/ 92 | 4/ 12 | 12/ 20 | 11/ 17 | 4/ 5 | 2/ 2 |
| Total (studies combined) | 90/143 (63%) | 22/ 41 (54%) | 50/ 95 (53%) | 45/ 60 (75%) | 13/ 31 (42%) | 7/ 10 (70%) |

*No. of infections with exacerbations/no. of all infections observed

the present study are in agreement with a combination of those of previous reports, except for influenza B and adenoviruses, for which present rates are lower and higher, respectively. This could be due to differences in clinical symptoms of these infections, as severity of respiratory infection symptoms is related to exacerbation rate.

# CHAPTER 12
# THEORETICAL DISCUSSION

## 12-1 Introduction

The mechanisms by which viral infections are related to the cause and course of CNSLD are not completely understood. Nevertheless, there are many reports on the subject indicating that several pathogenetic mechanisms might be involved. Especially bronchial hyperreactivity, which is a major feature in patients with CNSLD[19 52 91 274 286], seems to play an important role in virus-induced exacerbations of the disease. In this chapter a summary of the most important studies in this field will be given. Furthermore, some hypothetical considerations based on the results of the survey, described in this thesis, will be presented.

## 12-2 Studies in healthy subjects

It is worthwhile looking at studies indicating that acute viral and mycoplasmal infections affect lung function and the state of bronchial reactivity to various stimuli in normal subjects, since subtle effects might easily be overlooked in the presence of advanced disease. Moreover, the study of hyperreactivity in patients is complicated by hypertrophy and hyperplasia of airway smooth muscle, treatment with drugs and variable degrees of airway obstruction, making it difficult to estimate virus-induced changes in this respect.

Stonehill *et al.*[252] and Johansen *et al.*[131] were the first to report on minor restrictive spirometric changes in some subjects with acute viral respiratory disease. Picken *et al.*[218] and Blair *et al.*[16] found frequency dependency of dynamic compliance in otherwise healthy persons after clinically mild respiratory infection. These changes were found 4-8 weeks after infection and a further 6 weeks were required for lung function to return entirely to normal.

Reversible alterations in closing volume and He-$O_2$ flow rates at low lung volumes in smokers with rhinovirus infections were reported by

Fridy *et al.*[82] Prolonged reductions in carbon monoxide diffusing capacity have been described after infection by influenza[118], *Mycoplasma pneumoniae*[15], and rhinovirus.[33]

The results of these studies have in common that they are consistent with lower respiratory tract involvement in the course of viral illness in healthy persons. There are several reports indicating that in healthy human subjects viral respiratory infections cause a transient but sometimes striking increase in the bronchial response to various stimuli. Parker *et al.*[210] observed an enhanced response to metacholine in normal subjects during and after acute respiratory infections. Similarly, transient increased bronchial reactivity to histamine in subjects with colds was reported by Empey *et al.*[68] and after successful inoculation of live attenuated influenza virus by Laitinen *et al.*[146] In another study by Little *et al.*[160] a heightened responsiveness to inhaled carbachol was notified. Recently, it has been demonstrated that during upper respiratory infection normal subjects develop transient airway hyperreactivity to exercise with cold, dry air.[6] In healthy subjects bronchial reactivity is seen to return to control levels within 6 to 8 weeks.[6 68 146 160 210]

The most plausible explanation for the observed changes in bronchial reactivity is presented by Empey *et al.*[68] They provide evidence that the epithelial damage, known to occur in respiratory viral infection [111 113] sensitizes the rapidly adapting irritant receptors causing an increase in bronchomotor response to inhaled histamine through cholinergic, vagal pathways. Prior inhalation of atropine aerosol blocked the increase in responsiveness, an observation confirmed by Aquilina *et al.*[6] Recently, the importance of vagal pathways in the increased bronchial responsiveness to histamine in respiratory infection has been confirmed again in an experimental study by Dixon *et al.*[58]

12-3 Studies in CNSLD patients

The study of Parker *et al.*[210], already mentioned, indicated that in patients with bronchial asthma a heightened responsiveness to various stimuli exists in the course of respiratory infection as compared to their baseline hyperreactivity. There are some reports in which it is

suggested that influenza and measles vaccination have similar effects. A significant increase in sensitivity to inhaled metacholine after influenza vaccination, administered by subcutaneous injection, was notified in 9 out of 10 asthmatics by Ouellette and Reed[208] and in 11 out of 24 patients by Anand *et al.*[5] A similar effect after inoculation of live measles vaccine was reported by Kumar *et al.*[144], cited by Chai.[35] More recently, Laitinen and Kava[147] demonstrated an increased response to histamine in asthmatics but not in control persons following administration of influenza A virus by nasal drops. The heightened response was blocked by prior administration of both salbutamol and ipratropium bromide.

Studies with regard to vaccination in patients classified as chronic bronchitics are rare. There is one observation of increased respiratory symptoms with slight lung function alterations 3 weeks following local influenza vaccination.[301] The above-mentioned studies by Ouellette and Reed[208] and Anand *et al.*[5] bear important theoretical implications, as these investigators used vaccines given by injection in order to avoid bronchial epithelial damage. Despite the absence of local irritation, significant increases in bronchial reactivity were found. This means that in patients with asthma there must be some mechanism apart from epithelial damage capable of changing bronchial responsiveness. Allergy to viral products has been propagated as a possible explanation[66 104], but there is little evidence to support this hypothesis.[114] Some recent data suggest that chemical mediators such as histamine are involved in virus-induced wheezing. Ida *et al.*[123] have demonstrated that incubation of human leucocytes from atopic individuals with virus *in vitro* enhances their histamine release when they are subsequently exposed to ragweed antigen or antihuman IgE. Welliver *et al.*[290] reported that continued presence of cell-bound IgE was more common in patients with RSV-induced bronchiolitis or asthma than in patients with pneumonia or upper respiratory tract infection caused by RSV. Persistence of IgE was also related to the family history of wheezing. Thus local production of IgE in the respiratory tract and subsequent release of histamine may contribute to the clinical outcome of respiratory illness from infection by RSV.

Szentivanyi[264] introduced the idea of beta-adrenergic receptor

blockade as the mechanism underlying bronchial asthma, and his theory has recently been put forward again in a slightly modified way.[265] This hypothesis is based, in part, on the observation that mice vaccinated with *Bordetella pertussis* became hypersensitive to histamine and other mediators of anaphylaxis.[143] Infection was postulated to be the most important factor leading to blockade of beta-adrenergic receptors.

Subsequent investigations have shown that beta-adrenergic responsiveness of granulocytes is impaired by respiratory viral infection[27 28 29] and this observation provides a possible explanation for virus-induced asthmatic exacerbations.

12-4 Hypothetical considerations

One of the most important findings of the study described in this thesis is the existence of different patterns of reaction to viral respiratory infection in patients with various clinical entities covered by the term CNSLD. Although exacerbation rates did not differ significantly between patients with asthma and those with chronic airflow obstruction, the asthmatic subjects had increases of bronchial obstruction, confirmed by $FEV_1$ measurements, more frequently. On the contrary, the subjects with chronic airflow obstruction were more often inclined to expectorate coloured sputum in the course of SRI of viral and unknown origin. Patients with features of asthma as well as chronic airflow obstruction showed reaction patterns compatible with those of both conditions.

In our opinion the bronchial reaction to respiratory viruses is determined by a complex interaction between infecting agent and host response. Viral characteristics such as *in vitro* pathogenicity, the destructive effect at the cellular level, and the affinity for certain areas of the respiratory tract play an important role.[169]

On the other hand, host factors have been shown to be of the utmost importance in determining the clinical outcome of viral respiratory infection.[94 116] This observation is in agreement with our data.

It is tempting to speculate on the possible mechanisms underlying the differences in reaction patterns that were observed in the present study.

The above-mentioned hypothesis of increased cholinergic activity caused by epithelial damage[68] is well established and it accounts for

one of the possible factors involved in alterations in bronchial hyperreactivity seen both in healthy and CNSLD subjects. However, in our opinion this theory is not sufficient to explain the data described in this thesis completely. Additional mechanisms must be involved in order to clarify the different patterns of reaction that were observed. Gökemeijer[91] found differences in bronchial hyperreactivity profiles between a group of asthmatics and a group of chronic bronchitics. In general, the bronchitics were less hyperreactive. Furthermore, their pattern was supposed to be determined mainly be an increased cholinergic activity, whereas in the asthma-group decreased responsiveness of the sympathicoadrenergic receptors was thought to be the central disturbance. Other studies have also demonstrated that beta-adrenergic function disturbances are distinct in subjects with asthma[24] , whereas chronic bronchitics have only moderate reduction in responsiveness[155] or none at all.[130]

These observations and the results of some of the studies mentioned in chapter 12-3[5 27 28 29 208] are suggestive of a beta adrenergic blockade to be one of the fundamental mechanisms underlying the sometimes severe bronchial obstructive reactions notified in the group of asthmatics. Increased cholinergic activity is an additional important background in this respect.[6 68 197]

We suppose that the reaction patterns in the group of patients with chronic airflow obstruction are determined by factors other than beta-receptor blockade. Impairment of local defensive mechanisms, such as mucociliary clearance, macrophage function[178] and secretory-IgA production[245] together with mucous hypersecretion per se[73] may be possible pathogenetic conditions in this respect.

In the transitory clinical state with features of both asthma and chronic airflow obstruction, often referred to as asthmatic bronchitis, involvement of a combination of the above-mentioned mechanisms is likely to occur.

Fig. 12-1 shows a schematic representation of these hypothetical considerations. The survey described in this thesis can be regarded as a clinical field study into the effects of viral infection in patients with CNSLD. Further investigations, for example studies on the effects of inhalation of attenuated viral material on lung function and bronchial

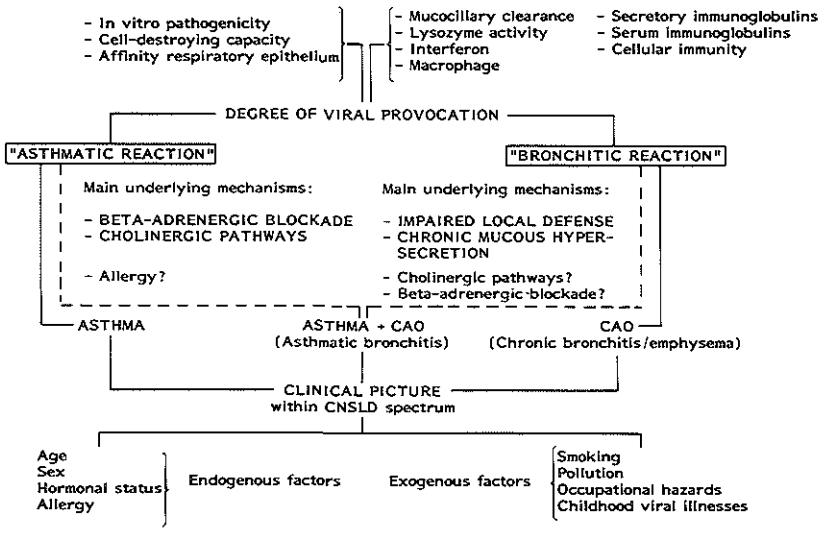


Fig. 12-1 Schematic representation of possible factors determining the final reaction patterns in CNSLD patients with respiratory viral infection

hyperreactivity, are needed to elucidate the exact mechanisms underlying the observed phenomena.

Simultaneously, research efforts toward the development of effective viral vaccines have to be stimulated in order to obtain possibilities to protect subjects with CNSLD against the adverse effects of viral respiratory infection.

## SUMMARY

This thesis describes the results of an investigation into the effects of respiratory infections caused by viruses, *Mycoplasma pneumoniae* and *Chlamydia psittaci* in a group of patients with chronic non-specific lung disease (CNSLD) during their stay in the Netherlands Asthma Centre Davos, situated at 1560 m above sea level. After a short introduction in which the aims of the study are summarized by formulating six questions, part I deals with general information and literature data regarding CNSLD, respiratory virology, effects of respiratory infection on cause and course of CNSLD, and effects of climatic factors in Davos.

Chapter 1 reviews the terminology applied for conditions attended with breathlessness and wheezing, cough, and expectoration of sputum. In The Netherlands, the general opinion is that these conditions should be grouped and the name "Chronische Aspecifieke Respiratoire Aandoeningen" (CARA = CNSLD) has become a well adapted omnibus term for these. The arguments in favour of this term, put forward especially by the Groningen workgroup of Orie and associates, are described in this chapter and the necessity of characterizing each individual patient according to a number of parameters is mentioned. Subsequently, attention is given to the different diagnoses commonly used in Anglo-Saxon literature, such as bronchial asthma, chronic bronchitis, and pulmonary emphysema. A brief description of these clinical syndromes as well as a concise comment on bronchiectasis are given.

In chapter 2 general remarks are made with regard to the respiratory agents that were studied in the survey. After a short introduction, the characteristics and use of different laboratory methods are discussed for adenovirus, influenza, and parainfluenza viruses, and RSV, as well as the non-viral agents *Mycoplasma pneumoniae* and *Chlamydia psittaci*. Brief comments are made on the association of these microorganisms with human illness.

Chapter 3 summarizes literature data on the role of respiratory

infection in the pathogenesis of CNSLD. The importance of respiratory tract illnesses in infancy and early childhood in this respect is especially emphasized in paragraphs on subglottic laryngotracheobronchitis (croup) and bronchiolitis. These conditions have been shown to be associated with a subsequent heightened bronchial reactivity to various stimuli, which is a major pathogenetic hallmark in bronchial obstructive disorders. Several studies indicate that respiratory infection can act as a trigger mechanism for the appearance of symptoms in subjects genetically predisposed to develop signs and symptoms of CNSLD.

In chapter 4 a review is given of literature dealing with the effects of respiratory viral infections on the clinical course of CNSLD. The majority of patients with bronchial obstructive diseases tend to react with an exacerbation in the course of viral infection of the respiratory tract. However, literature data are very divergent, mainly because of the absence of uniform definitions of SRI and exacerbation. As most reports deal with patients categorized as asthmatics or chronic bronchitics only, literature data are discussed for these groups separately.

Subsequently, the effects of climatic factors and the situation of the Netherlands Asthma Centre are described. In chapter 5 three theories on the favourable influence of a high mountain climate are discussed, i.e.: low density of inspired air, biometereological conditions, and relative absence of certain allergens, especially those produced by the house-dust mite, *Dermatophagoides pteronyssinus*. The clinic's model of treatment, strongly directed toward physical and mental rehabilitation, is presented for 3 different groups of patients in chapter 6.

Part II of this thesis deals with the design of the study and the results.

In chapter 7 the patients participating are categorized according to living-unit and mainly based on age, into group A - "Senioren", group B - "Junioren", and group C - "Vossen". Moreover, a classification is made of clinical categories on the base of anamnestic data and reversibility of bronchial obstruction, measured by lung function tests. Thus, 3 groups are formed: group I - asthma (good reversibility at lung function), group II - asthma and chronic airflow obstruction (moderate reversibility), and group III - chronic airflow obstruction (CAO) (poor

reversibility). Table 7-6 shows the population of patients classified according to living-unit, clinical picture, and sex. In the course of the study daily surveillance was carried out for each patient. Special attention was given to the occurrence of symptomatic respiratory infection (SRI), according to criteria described in chapter 7-3-1. Scores for dyspnoea/wheezing and cough/sputum production, as well as measurements of $FEV_1$ were noted daily for each patient. Criteria for exacerbation of CNSLD are given in chapter 7-3-2. In case of SRI, serological investigations of paired blood samples were done using the following antigens: influenza A, influenza B, parainfluenza 1, 2, and 3 viruses, adenovirus, RSV, *Mycoplasma pneumoniae* and *Chlamydia psittaci*. Furthermore, sera were tested routinely at 4-week intervals in order to check whether any subclinical infections had occurred.

If yellow or green coloured sputum was expectorated in the course of SRI, a gram stain of a carefully washed flake was made. Additional laboratory investigations that were carried out are described in chapter 7-3-3.

In chapter 8 the results of the survey are presented for living-groups A, B, and C. Group A patients experienced 127 episodes of SRI with a rate of 0.18 SRI per patient month. Exacerbation was noted in 38 out of 44 SRIs with positive serology (Table 8-5) and in 53 out of 81 SRIs without positive serology (Table 8-7). Green or yellow coloured sputum was expectorated in 25 and 41 of these SRIs, respectively. Seroconversion without SRI could be related to exacerbation in 6 out of 15 episodes with mild symptoms (MRI = mild respiratory infection) and in none of the 6 subclinical infections (Table 8-8).

In group B, 98 SRIs were found with a rate of 0.16 SRI per patient month. Twenty-seven SRIs were related to seroconversion, 20 of these with exacerbation (Table 8-13). Coloured sputum was reported in 13 instances. Fifty out of 71 remaining SRIs were associated with exacerbation (Table 8-15). Yellow/green sputum was noted in 46 subjects. Exacerbation was observed in 5 out of 12 MRIs with seroconversion and in 2 out of 7 subclinical infections (Table 8-16).

Group C patients reported 59 SRIs in 465 patient months, equal to a rate of 0.13 . Exacerbation was observed in 16 out of 18 SRIs with

positive serology (Table 8-21) and coloured sputum was expectorated 13 times. In SRI without positive serology streptococcal pharyngitis was diagnosed in 5 subjects, none of them with any sign of exacerbation. A flare-up of CNSLD was noticed in 23 out of the remaining 35 SRIs (Table 8-23). Sixteen patients coughed up coloured sputum. MRI could be related to exacerbation in 4 out of 12 and subclinical infection in 2 out of 9 subjects (Table 8-24). In chapter 8-5 these results are combined.

Chapter 9 summarizes the results for patients of different clinical groups I, II, and III. Although infection rates and incidence of exacerbation do not differ significantly, it is shown that SRI gives rise to different patterns of exacerbation in these subgroups of CNSLD patients. Subjects categorized in group I (asthma) react with marked bronchial obstruction, whereas group III patients (CAO) tend to react with increasing cough and sputum production. Patients of group II (asthma + CAO) have features of both phenomena. These data are shown in Tables 9-15 to 9-19.

The effects brought about by the various microorganisms tested for in the study, are summarized in chapter 10. For each agent epidemiology and clinical picture as well as effects on CNSLD parameters in SRI and seroconversion without SRI are described. Infections by all agents except influenza B virus could be related to exacerbation in a majority of instances. The results are shown in Table 10-10.

In chapter 11 the results of the present study are compared to literature data. SRI rates in asthma are difficult to compare because of differences in age of various populations studied, whereas frequency of SRI in CAO is similar to that reported for chronic bronchitis by others. Figures of viral identification in SRI are similar to those found in other studies. The exacerbation rate of viral infection in asthma in the present study is the highest reported hitherto, while the frequency of exacerbation in CAO does not differ from other reports. This is probably due to the attention given to more than one parameter in rating exacerbations. Especially in groups I and II, daily $FEV_1$ measurement turned out to be a valuable parameter. The frequency of bacterial sputum infection in SRI as judged by gram stain preparations, is in

agreement with previous reports.

In chapter 12 some theoretical contemplations on the background of CNSLD exacerbation in respiratory viral infection are presented. A hypothesis is put forward emphasizing the importance of beta-adrenergic blockade being one of the central mechanisms in virus-induced exacerbations in asthma. The phenomena observed in patients with chronic airflow obstruction do not seem to depend on beta-adrenergic blockade, but rather on impaired local defense mechanisms and chronic mucous hypersecretion. Fig. 12-1 is a schematic representation of these hypothetical considerations.

## SAMENVATTING

In dit proefschrift worden de resultaten beschreven van een onderzoek naar de gevolgen van luchtweginfecties veroorzaakt door een aantal virussen, *Mycoplasma pneumoniae* en *Chlamydia psittaci* bij een groep patiënten met chronische aspecifieke respiratoire aandoeningen (CARA) tijdens hun verblijf in het Nederlands Astmacentrum Davos, gelegen op 1560 m hoogte. Na een korte inleiding, waarin het doel van het onderzoek door het formuleren van een zestal vragen wordt uiteengezet, worden in deel I algemene informatie en literatuurgegevens betreffende CARA, respiratoire virologie, de betekenis van luchtweginfecties bij CARA, alsmede de invloed van de klimatologische omstandigheden in Davos samengevat.

In hoofdstuk 1 wordt ingegaan op de terminologie van de aandoeningen waarbij kortademigheid en piepen op de borst, hoesten en opgeven van sputum de symptomen vormen. In Nederland is de algemene opvatting dat deze aandoeningen onder één noemer gebracht dienen te worden en de term CARA is in dit verband een algemeen aanvaarde benaming geworden. De argumenten hiervoor, zoals die met name door de Groningse groep van Orie en medewerkers naar voren zijn gebracht, worden besproken en de noodzaak iedere patiënt individueel aan de hand van een aantal parameters te karakteriseren wordt vermeld. Vervolgens krijgen de veelal in de Engelstalige literatuur gebruikte diagnoses asthma bronchiale, chronische bronchitis en longemfyseem enige aandacht. Deze klinische beelden, alsmede het begrip bronchiectasie, worden kort besproken.

Hoofdstuk 2 is gewijd aan de in het onderzoek bestudeerde verwekkers van luchtweginfecties. Na een korte inleiding worden enige eigenschappen en laboratoriumtechnieken besproken van achtereenvolgens adenovirussen, influenza virussen en parainfluenza virussen, RSV en de niet-virale verwekkers *Mycoplasma pneumoniae* en *Chlamydia psittaci*. De relatie tussen infectie met deze agentia en de kliniek wordt beknopt becommentarieerd.

Hoofdstuk 3 vat de belangrijkste literatuurgegevens samen betreffende de rol van luchtweginfecties in de pathogenese van de CARA. Het belang van luchtwegaandoeningen in de vroege jeugd in dit opzicht wordt benadrukt in gedeelten over laryngotracheobronchitis (croup) en bronchiolitis. Er is aangetoond dat deze syndromen veelal in verband kunnen worden gebracht met het optreden van de voor CARA kenmerkende hyperreactiviteit van de luchtwegen op latere leeftijd. Diverse onderzoekingen geven aan dat luchtweginfectie als triggermechanisme kan optreden voor het verschijnen van symptomen bij daarvoor genetisch gepredisponeerde personen.

In hoofdstuk 4 wordt een literatuuroverzicht gegeven van de gevolgen van respiratoire infecties voor het verloop van de CARA-symptomen. Patiënten met CARA reageren in een merendeel der gevallen op een virale infectie met een exacerbatie van hun aandoening. De literatuurgegevens zijn echter niet eensluidend, hoofdzakelijk omdat geen uniforme definities bestaan voor begrippen als symptomatische respiratoire infectie (SRI) en exacerbatie. Aangezien de meeste publicaties betrekking hebben op patiënten met asthma bronchiale of chronische bronchitis, wordt de literatuur voor deze groepen afzonderlijk samengevat.

Vervolgens worden de klimatologische omstandigheden van het hooggebergte en de situatie in het Nederlands Astmacentrum beschreven. In hoofdstuk 5 worden drie theorieën besproken die omtrent de voor CARA gunstige werking van het hooggebergte zijn opgesteld, t.w. de lage dichtheid van de inademingslucht, de biometereologische omstandigheden en het in geringe mate voorkomen van bepaalde allergenen, vooral die geproduceerd door de huisstofmijt, *Dermatophagoides pteronyssinus*.

Het behandelingsmodel van het Nederlands Astmacentrum, sterk gericht op fysieke en mentale revalidatie, wordt in hoofdstuk 6 aan de hand van 3 verschillende groepen patiënten naar voren gebracht.

In deel II van dit proefschrift worden de opzet en de verkregen resultaten van het eigen onderzoek behandeld. In hoofdstuk 7 wordt een onderverdeling van de patiënten gemaakt aan de hand van de verschillende woonafdelingen waarbinnen de diverse leeftijdsgroepen zijn ondergebracht. Aldus worden de groepen A - Senioren, B - Junioren en C - Vossen onderscheiden. Bovendien worden de deelnemers geclassificeerd in verschillende klinische categorieën op grond van anammestische gegevens en de bij

longfunctieonderzoek gemeten reversibiliteit van luchtwegobstructie. Op deze wijze worden 3 groepen gevormd: groep I - astma (grote reversibiliteit), groep II - astma en chronische obstructieve luchtwegaandoening (matige reversibiliteit) en groep III - chronische obstructieve luchtwegaandoening (geringe reversibiliteit). Tabel 7-6 toont de patiëntenpopulatie, verdeeld volgens woonafdeling, klinisch beeld en geslacht. Tijdens de studieperiode werd een aantal parameters voor iedere patiënt dagelijks vastgelegd. Op het vóórkomen van verschijnselen van een luchtweginfectie ("symptomatic respiratory infection" = SRI) werd bijzonder gelet, overeenkomstig de in hoofdstuk 7-3-1 beschreven criteria. Scores voor kortademigheid/piepen en hoesten/sputum opgeven en meting van het expiratoire 1-seconde volumen werden dagelijks geregistreerd. De criteria, volgens welke het begrip exacerbatie werd omschreven, worden weergegeven in hoofdstuk 7-3-2. Indien een SRI optrad werd serologisch onderzoek van gepaarde bloedmonsters verricht met de volgende antigenen: influenza A en B virussen, parainfluenza 1, 2 en 3-virussen, adenovirus, respiratoir syncytieel virus, *Mycoplasma pneumoniae* en *Chlamydia psittaci*. Voorts werd routinematig serologisch onderzoek verricht van iedere 4 weken verkregen sera om na te gaan of subklinische infecties waren voorgekomen. Bij expectoratie van geel of groen gekleurd sputum tijdens een SRI, werd een grampreparaat van een zorgvuldig gewassen sputumvlok gemaakt. Aanvullend laboratoriumonderzoek, zoals dat werd verricht, wordt beschreven in hoofdstuk 7-3-3.

In hoofdstuk 8 worden de resultaten van het onderzoek gegeven voor de leefgroepen A, B en C. De patiënten in groep A rapporteerden 127 SRI's, hetgeen overeenkomt met 0,18 SRI per patiëntmaand. Een exacerbatie werd gezien tijdens 38 van de 44 SRI's met positieve serologie (tabel 8-5) en tijdens 53 van de 81 SRI's zonder positieve serologie (tabel 8-7). Expectoratie van groen of geel gekleurd sputum kwam voor bij respectievelijk 25 en 41 van deze SRI's. Titerstijgingen zonder SRI vielen samen met een CARA-exacerbatie bij 6 van de 15 perioden waarin op grond van zeer geringe luchtwegverschijnselen het tijdstip van infectie kon worden achterhaald ("mild respiratory infection" = MRI) en bij geen der 6 geheel symptoomloze infecties (tabel 8-8). In groep B werden 98 SRI's gevonden met een frequentie van 0,16 SRI per patiënt-

maand. Zevenentwintig SRI's gingen gepaard met een titerstijging; in 20 van deze perioden trad een exacerbatie op (tabel 8-13). In 13 gevallen werd gekleurd sputum opgehoest. Van de overige 71 SRI's trad bij 50 een exacerbatie van de CARA op (tabel 8-15). Geel/groen sputum werd geëxpectoreerd door 46 patiënten. Een exacerbatie werd geregistreerd in 5 van de 12 MRI's met titerstijging en in 2 van de 7 subklinische infecties (tabel 8-16). De patiënten in groep C gaven in 465 patiëntmaanden 59 maal het voorkomen van een SRI aan, hetgeen gelijk is aan 0,13 SRI per patiëntmaand. Bij 16 van de 18 SRI's met positieve serologie werd een exacerbatie gevonden (tabel 8-21) terwijl 13 maal gekleurd sputum werd opgegeven. Bij 5 kinderen met een SRI zonder titerstijging werd een streptococcen-faryngitis vastgesteld; in geen van deze episoden werd enig teken van exacerbatie gevonden. Een verergering van de CARA werd gezien tijdens 23 van de 35 resterende SRI's (tabel 8-23). Zestien patiënten hoestten tijdens een dergelijke periode gekleurd sputum op. Een exacerbatie werd geregistreerd tijdens 4 van de 12 MRI's en 2 van de 9 subklinische infecties (tabel 8-24). In hoofdstuk 8-5 worden de bovenstaande resultaten gecombineerd weergegeven.

Hoofdstuk 9 behandelt de resultaten voor de patiënten van de groepen I, II en III met verschillende klinische verschijningsvormen. Hoewel het vóórkomen van infecties en exacerbaties per groep niet significant verschillend is, is te zien dat symptomatische luchtweginfecties aanleiding geven tot verschillende uitingsvormen van exacerbatie bij deze subgroeperingen van CARA-patiënten. Patiënten, geclassificeerd in groep I (astma) reageren met uitgesproken luchtwegobstructie, terwijl de patiënten van groep III (chronische obstructieve luchtwegaandoening) meer geneigd zijn tot vermeerderd hoesten en expectoreren van sputum. Patiënten van de overgangsgroep II hebben een combinatie van bronchusobstructie en productief hoesten. Deze gegevens worden weergegeven in de tabellen 9-15 tot en met 9-19.

De gevolgen van infectie met de verschillende in het onderzoek bestudeerde microörganismen worden samengevat in hoofdstuk 10. De epidemiologische en klinische bevindingen van infectie met de diverse agentia, alsmede de gevolgen op de CARA-parameters in het geval van SRI en titerstijging zonder SRI, worden beschreven. Voor alle agentia, behalve

influenza B, werd gevonden dat infectie in het merendeel der gevallen gepaard ging met een exacerbatie van de CARA. De resultaten worden vermeld in tabel 10-10.

In hoofdstuk 11 worden de resultaten van het onderhavige onderzoek vergeleken met uit de literatuur bekende gegevens. De SRI-frequentie bij asthmatici is moeilijk met die van andere studies te vergelijken vanwege verschillen in de leeftijdsopbouw van de diverse bestudeerde patiëntenpopulaties, terwijl de frequentie van SRI in het geval van chronische obstructieve luchtwegaandoeningen overeenkomt met gegevens van andere onderzoekers voor chronische bronchitis. Het percentage SRI's met een geïdentificeerde verwekker is overeenkomstig de getallen van andere publicaties. Voor de astma-groep is het aantal virusinfecties dat tot exacerbatie aanleiding geeft het hoogst in de literatuur vermelde tot nu toe, terwijl voor de obstructieve luchtwegaandoeningen met irreversibele afwijkingen geen duidelijke verschillen met andere studies gevonden worden. Dit is waarschijnlijk het gevolg van het feit dat bij het beoordelen van het begrip exacerbatie rekening werd gehouden met meer dan één parameter. Vooral voor de groepen I en II bleek het dagelijks registreren van het expiratoire 1-seconde volumen een waardevol meetinstrument. De frequentie van het optreden van bacteriële sputuminfecties bij SRI's is in overeenstemming met de literatuurgegevens.

Hoofdstuk 12 is gewijd aan enige theoretische overwegingen betreffende de achtergronden van het optreden van door virusinfecties geïnduceerde exacerbaties bij CARA-patiënten. Het belang van beta-adrenerge receptor blokkering als een van de centrale mechanismen bij exacerbaties van astma tijdens virale infecties wordt als een hypothese naar voren gebracht. De verschijnselen zoals die gezien worden bij patiënten met chronische obstructieve luchtwegaandoeningen lijken niet gebaseerd te zijn op beta-adrenerge blokkade, maar eerder op beschadiging van locale afweermechanismen en chronische hypersecretie. Fig. 12-1 is een schematische afbeelding van deze hypothetische overwegingen.

# NAWOORD

Mijn dank gaat uit naar allen, die een - al dan niet bescheiden - steentje hebben bijgedragen aan het tot stand komen van dit proefschrift. Bijzondere erkentelijkheid ben ik verschuldigd aan onderstaande personen.

*Prof.Dr.N.Masurel*, die het onderzoek vanaf het prille begin op een uiterst stimulerende wijze begeleidde en mij bij herhaling over de bekende dode punten bij het verzamelen en bewerken van de gegevens heen tilde.
*Regina Engels-Bakker*, zijn secretaresse, die met een onnavolgbare persoonlijke inzet niet alleen het manuscript enige malen uittypte, de lay-out en de literatuurlijst verzorgde, maar daarnaast gedurende de afgelopen zes jaar in vele aangelegenheden met betrekking tot dit proefschrift mijn steun en toeverlaat was.
*Prof.Dr.N.G.M.Orie*, die mij met zijn grote ervaring op het gebied van de CARA vele malen van dienst was en mij behoedde voor al te vrijpostige interpretaties van de verkregen gegevens.
*Prof.Dr.K.F.Kerrebijn*, *Prof.Dr.J.Desmyter* en *Dr.A.Löwenberg* voor het kritisch doorlezen en becommentariëren van het manuscript.
De *medewerkers van de Afdeling Virologie*, Erasmus Universiteit Rotterdam, voor hun aandeel in het virologisch laboratoriumwerk.
*Hubert Schouten*, afdeling Biostatistica, Erasmus Universiteit Rotterdam, voor hulp bij de statistische verwerking van de gegevens.
*Margreet van Donselaar*, *Karen de Bondt*, *Elsa Kraan*, *Truus* en *Ton van Essel*, *Yolande Louis*, *Evelien Bogaard*, *Mary van der Berg*, *Lody van Velthoven* en *Jos Tahapary* voor de vele laboratorium en administratieve werkzaamheden bij het verzamelen van de gegevens in Davos.
*Paul Zuidema* en *Chris de Jong* voor het regelmatig overnemen van mijn dagelijkse bezigheden in het Nederlands Astmacentrum, wanneer de werkzaamheden voor het proefschrift dit nodig maakten.
*Rudi Fueter* für seine Bemühungen bei der Beurteilung der Röntgenbilder.
*Mw.B.Sluys*, Bacteriologisch Laboratorium, Academisch Ziekenhuis

Groningen voor het herbeoordelen van de grampreparaten van het sputum.
*Jan Ernst Douma* voor het vervaardigen van het omslag-ontwerp.
*Bea Grashoff*, *Cees de Vries* en *overige medewerkers* van de Audiovisuele Dienst, Erasmus Universiteit Rotterdam, voor de uitvoering van de figuren.
Last but not least: *alle patiënten* voor hun bereidwilligheid aan het onderzoek deel te nemen en voor hun grote betrokkenheid bij het rijpingsproces van dit proefschrift.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| CA | Croup-associated |
| CARA | "Chronische aspecifieke respiratoire aandoeningen" |
| CAO | Chronic airflow obstruction |
| CF | Complement fixation |
| CNSLD | Chronic non-specific lung disease |
| COAD | Chronic obstructive airway disease |
| COLD | Chronic obstructive lung disease |
| COPD | Chronic obstructive pulmonary disease |
| C/S | Cough/sputum |
| D/W | Dyspnoea/wheezing |
| ECF-A | Eosinophil chemotactic factor of anaphylaxis |
| $FEV_1$ | Forced expiratory volume in 1 second |
| H | Haemagglutinin |
| HA | Haemadsorption |
| HI | Haemagglutination inhibition |
| MRI | Mild respiratory infection |
| N | Neuraminidase |
| PAF | Platelet activating factor |
| PPLO | Pleuro-pneumonia like organism |
| RSV | Respiratory syncytial virus |
| SRI | Symptomatic respiratory infection |
| SRS-A | Slow reacting substance of anaphylaxis |

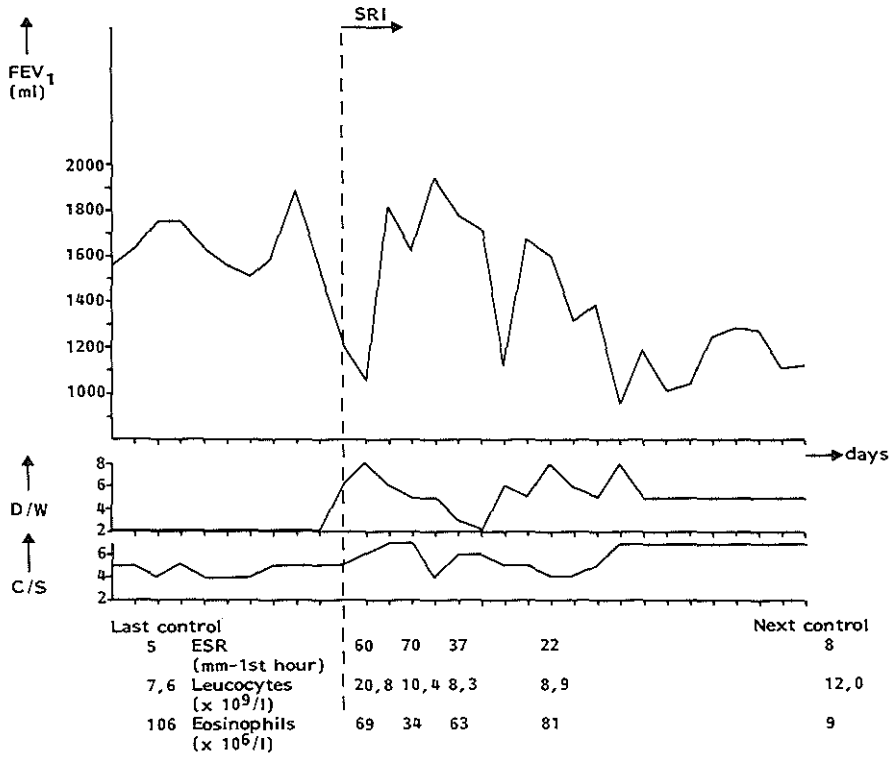
*CURRICULUM VITAE*

De schrijver van dit proefschrift werd op 14 mei 1949 te Rotterdam geboren. Na de lagere school bezocht hij in Rotterdam het Libanon Lyceum, alwaar in 1967 het diploma gymnasium-B werd behaald. In hetzelfde jaar werd een begin gemaakt met de studie geneeskunde aan de Medische Faculteit Rotterdam, later Erasmus Universiteit Rotterdam. In het kader van een keuzepracticum werd in 1970 gedurende 6 maanden wetenschappelijk onderzoek verricht in de toenmalige astmakliniek "Eugenia" in Davos, Zwitserland (directeur-geneesheer destijds: Dr.P.Zuidema). Na het behalen van het artsdiploma in november 1973 werd door middel van een opleiding in de huisartsgeneeskunde en een wisselassistentschap in een perifeer ziekenhuis ervaring in de practische geneeskunde opgedaan. De schrijver is sedert 1 juni 1975 werkzaam in het Nederlands Astmacentrum Davos, alwaar hij sinds september 1979 de functie van Hoofd Medische Dienst vervult.

# APPENDIX I

LEGEND TO CASE REPORTS:

$FEV_1$ = forced expiratory volume in 1 sec.
SRI = symptomatic respiratory infection
D/W = scores for dyspnoea/wheezing
C/S = scores for cough/sputum
ESR = erythrocyte sedimentation rate
MRI = mild respiratory infection



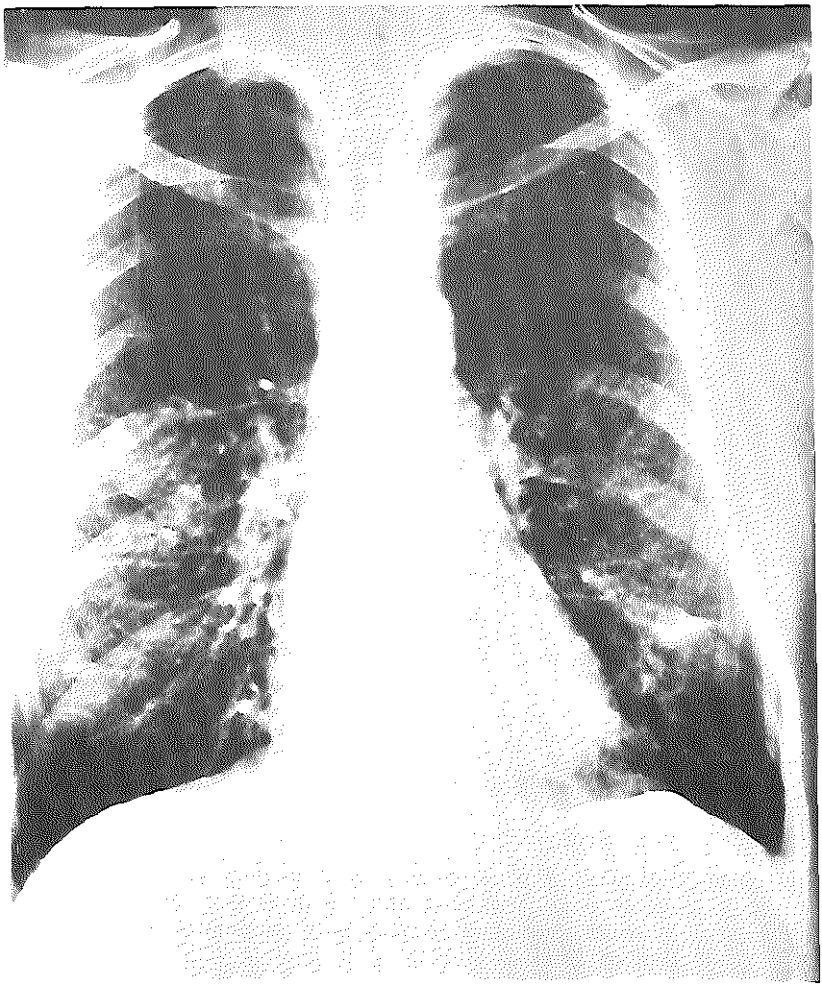

Case report 1

A48 Male 54 yrs Group A-II

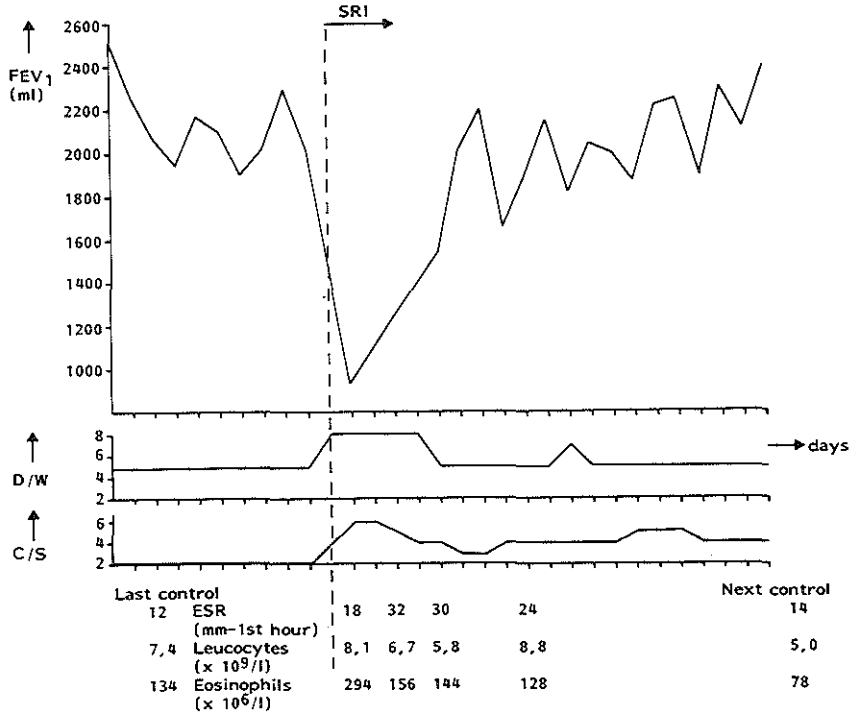
SRI symptoms: Malaise, dry coughing, hoarseness, headache, myalgia, chest pain. Malaise and fatigueness lasting for about 4 weeks. Mamixum rectal body temperature: 39.7° C on 2nd day of SRI.

Serology: Rise in CF titre to adenovirus from <7 to 32.

Asthma relatively well controlled before SRI. Less good control in the course of SRI and during the next 3 weeks, in spite of raising oral prednisolon from 15 mg to 40 mg daily. First discolouration of sputum on 2nd day of SRI. Sputum gram stain positive. Antibiotic treatment during 10 days of SRI and from 13th day onward. X-thorax: see page 196.



X-thorax patient A48 (case report 1). Triangular infiltrate in left lower part. Small spotty infiltrate in right middle section. Obliteration of left costo-diaphragmatic angle.



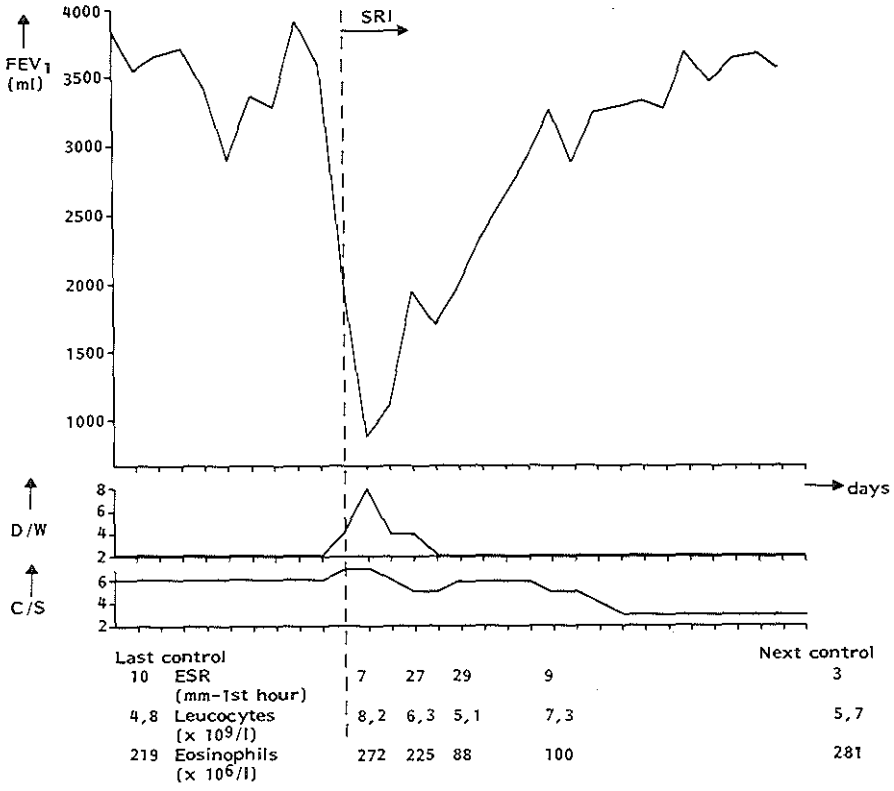

Case report 2

A25 Male 47 yrs Group A-I

SRI-symptoms: Malaise, sore throat, headache, myalgia, vomiting. Maximum rectal body temperature: 39.9° C on 1st day of SRI.

Serology: Rise in HI titre to influenza A/Texas/1/77 (H3N2) virus from 50 to 1950.

Asthma stable before SRI. Long-term corticosteroid therapy necessary: dosage before SRI 1.5 mg dexamethasone daily. Distinct bronchial obstructive reaction in the course of influenza A infection. No raise in steroid dosage. Return to pre-infection $FEV_1$ within 1 week. Prophylactic treatment with antibiotics. No discolouration of sputum. X-thorax unchanged.



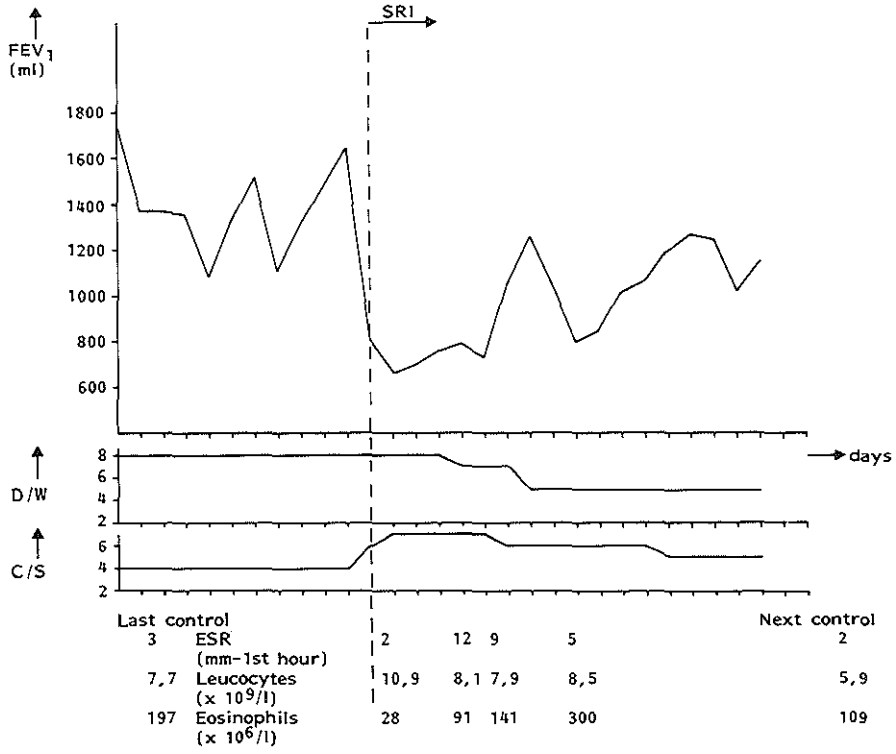

Case report 3

A74 Male 50 yrs Group A-II

SRI symptoms: Malaise, running and stuffed nose, sore throat, dry coughing, hoarseness, headache, myalgia, chest pain. Maximum rectal body temperature: 39.6° C on 2nd day of SRI.

Serology: Rise in CF titre to influenza A from <7 to 160.

Severe bronchial obstructive reaction following influenza A infection. Admission to intensive care unit, $FEV_1$ being 560 at noon on 1st day of SRI. Infusion treatment with corticosteroids and aminophylline. Return to pre-infection condition within 1 week. Slight discolouration of sputum during first 3 days of SRI. No gram stain made. No antibiotics. X-thorax unchanged.



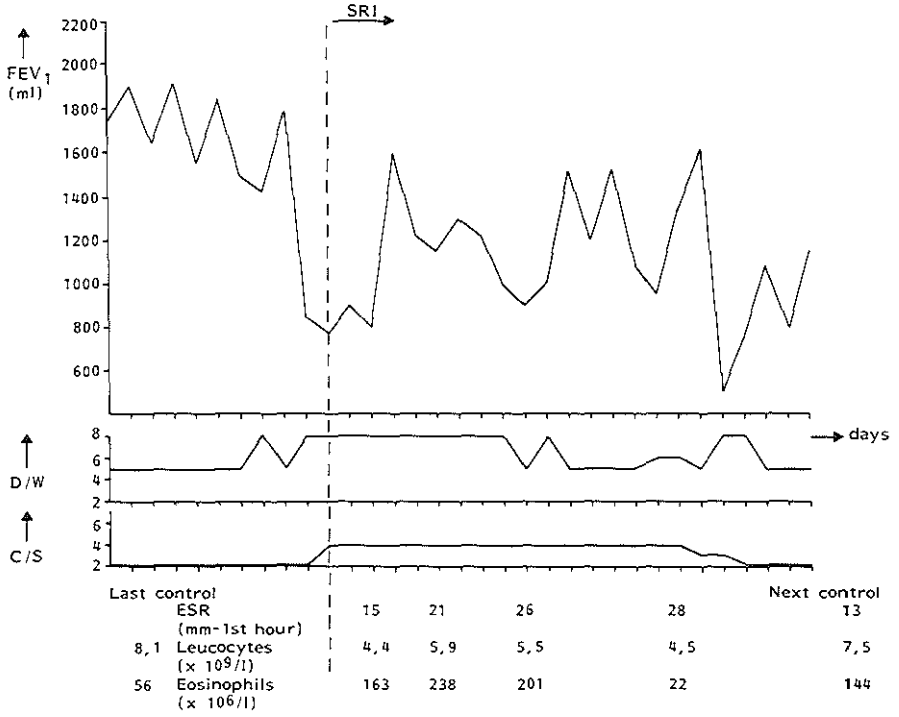

Case report 4

A55 Male 52 yrs Group A-I

SRI-symptoms: Malaise, running and stuffed nose, sore throat, hoarseness, headache, chest pain. Maximum rectal body temperature: 37.8° C during several days.

Serology: Negative

Asthmatic exacerbation in patient with fluctuating degrees of bronchial obstruction before SRI. Poor control of obstructive symptoms in spite of high dosages of predisolon -75 mg daily- and aminophylline. A second exacerbation occurred when prednisolon was gradually diminished to 20 mg daily after 3 weeks. Sputum colour changed from white to yellow-green on 1st day of SRI. Sputum gram stain positive. Antibiotic treatment was given. X-thorax unchanged.



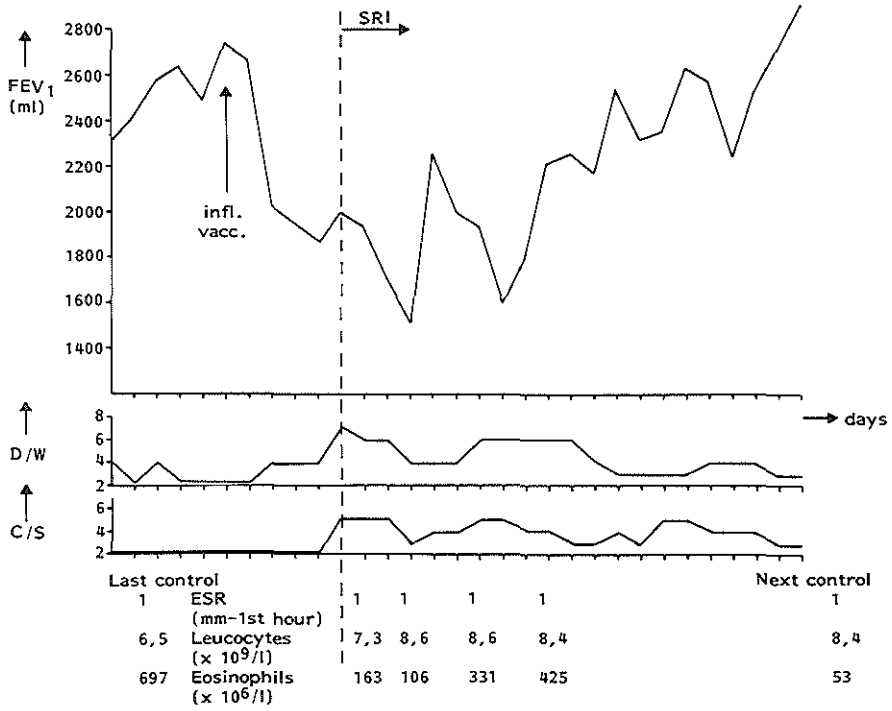

Case report 5

A67 Female 61 yrs Group A-I

SRI symptoms: Malaise, stuffed nose, hoarseness, headache, chest pain. Maximum rectal body temperature: 38.8° C on 2nd day of SRI.

Serology: Negative.

Asthma relatively well controlled. Increased bronchial obstruction starting 1 day before SRI. Poor control of asthma with large variations in $FEV_1$ in the course of SRI and several months afterwards. Only slight improvement after therapy with higher dosage of corticosteroids. No sputum expectoration. X-thorax unchanged.



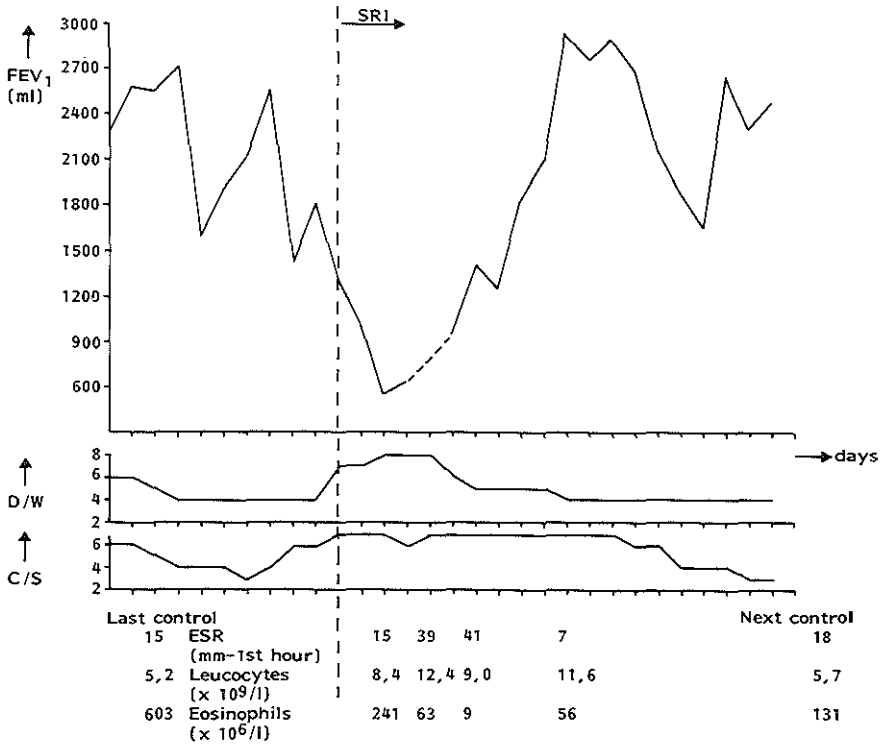

Case report 6

A33 Male 32 yrs Group A-I

SRI symptoms: Malaise, chest pain. Maximum rectal body temperature <37.8° C.

Serology: Negative.

SRI with expectoration of green, purulent sputum after some days of increased bronchial obstruction probably due to subcutaneous administration of influenza vaccine. Sputum gram stain was positive and cultures yielded growth of *Streptococcus pneumoniae* and *Haemophilus influenzae*. Good control of obstruction before vaccination. Return to pre-infection stable condition within 10 days. Corticosteroids were raised temporarily and antibiotics were administered.

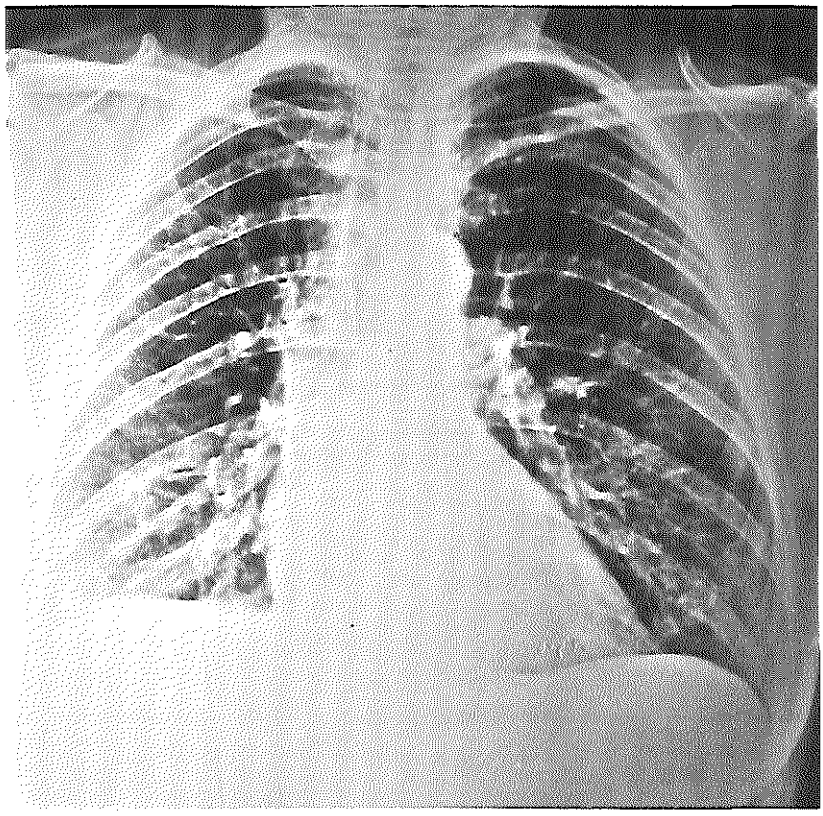



Case report 7

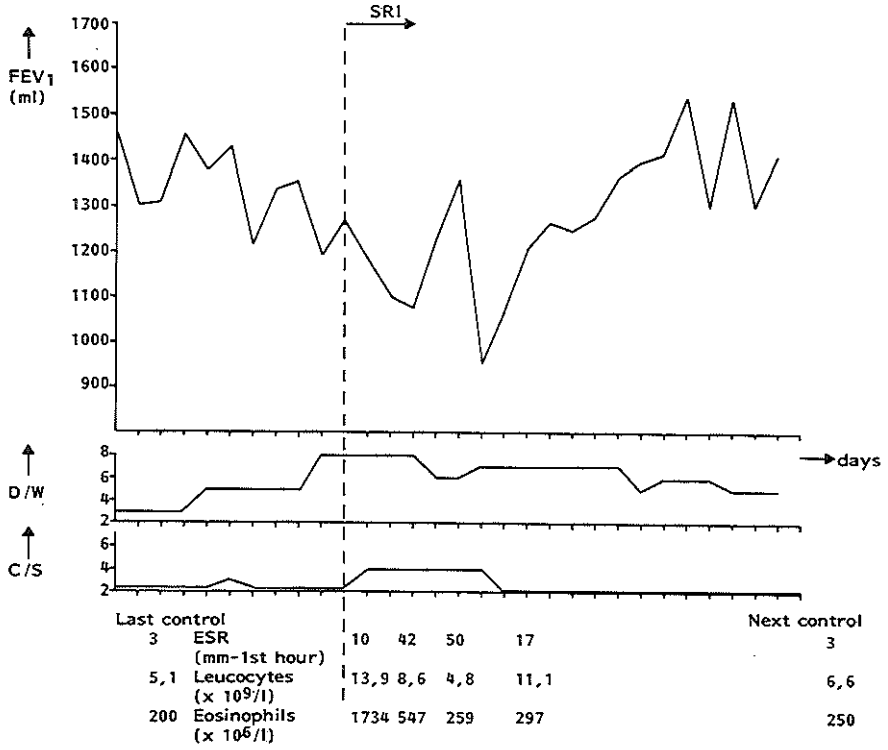
A3 Female 42 yrs Group A-I

SRI symptoms: Malaise, sore throat, running nose, sneezing, chest pain. Maximum body temperature: 37.2° C.
Serology: Negative.

Bronchial obstructive exacerbation for unknown reasons, followed by SRI associated with bacterial sputum infection: gram stain positive, *Streptococcus pneumoniae* in culture. Severe asthmatic reaction in the course of SRI, necessitating admission to intensive care unit and treatment with intravenous corticosteroids and aminophylline as well as administration of antibiotics. Recovery within a period of 10 days. X-thorax: see page 203.



X-thorax patient A3 (case report 7). Diffuse cloudy obliteration of right costo-diaphragmatic angle.



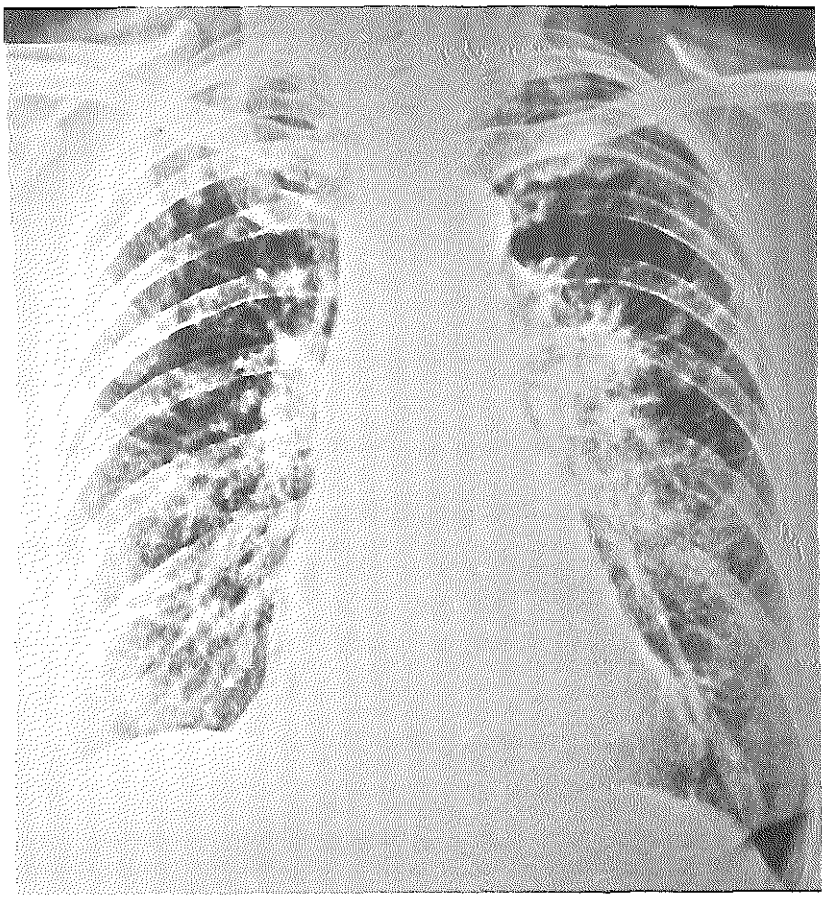

Case report 8

A57 Female 57 yrs Group A-II

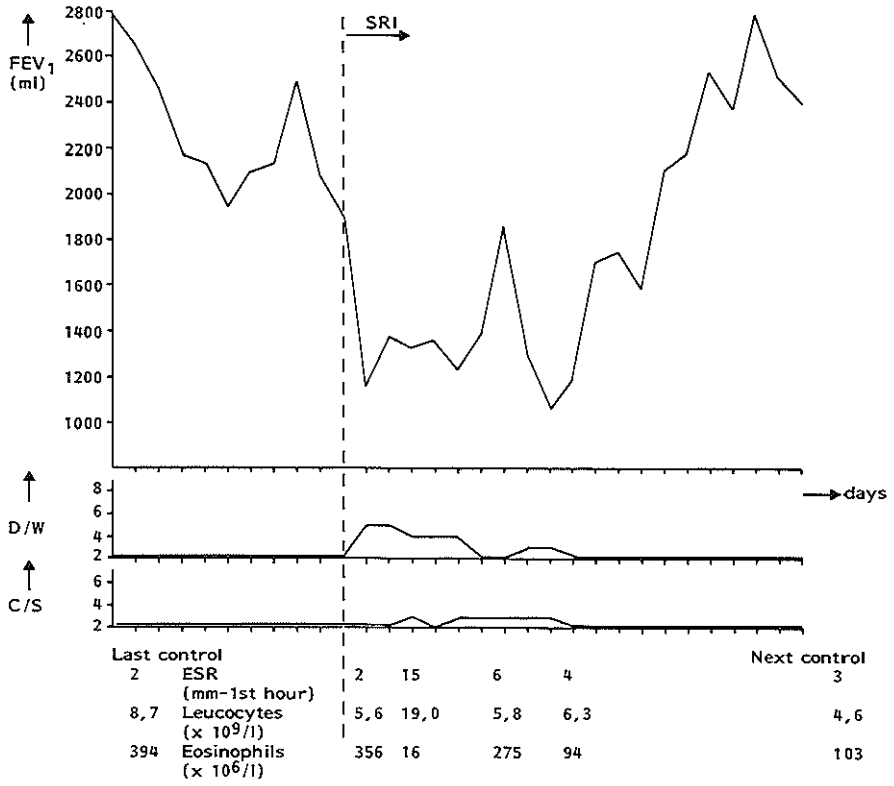
SRI symptoms: Malaise, stuffed nose, dry coughing, hoarseness, headache, myalgia, chest pain, vomiting, diarrhoea. Maximum rectal body temperature: 39.5° C on 1st day of SRI.

Serology: Negative.

Symptoms of SRI associated with fever and increase in total eosinophilic leucocyte counts. Expectoration of white-greyish sputum with abundant amounts of eosinophils. Institution of corticosteroids from 4th day of SRI onward gave gradual relief of signs and symptoms. These findings together with röntgenologic changes (X-thorax: see page 205) made the diagnosis "eosinophilic pneumonia" plausible.



X-thorax patient A57 (case report 8). Parahilar infiltrate in left midfield. Diffuse cloudy obliteration of right costo-diaphragmatic angle.



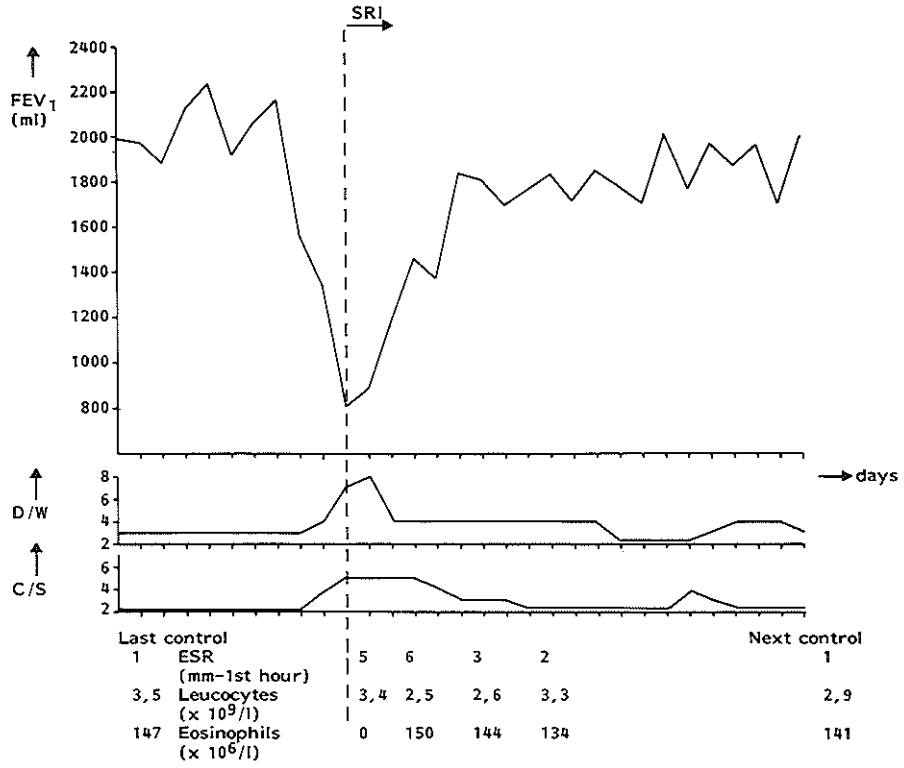

Case report 9

B17 Male 14 yrs Group B-I

SRI symptoms: Malaise, running nose, dry coughing, headache, sore throat, sore ears. Maximum rectal body temperature: 39° C on 3rd day of SRI.

Serology: Rise in CF titre to parainfluenza 3 from <7 to 770.

Distinct asthmatic reaction related to parainfluenza 3 infection with upper respiratory symptoms. Relatively prolonged exacerbation with spontaneous return to pre-infection condition within 2 weeks. No sputum expectorated.



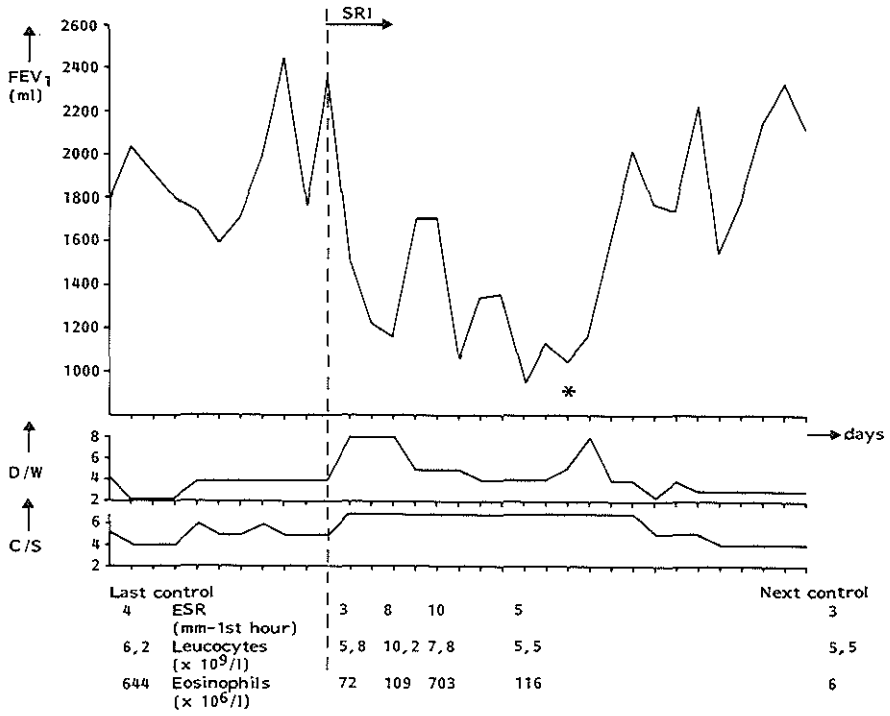

Case report 10

B28 Male 18 yrs Group B-II

SRI symptoms: Malaise, dry coughing, headache, vomiting. Maximum rectal body temperature: 39° C on 3rd day of SRI.

Serology: Rise in CF titre to parainfluenza from 7 to 160.

Marked increase of bronchial obstruction associated with SRI by parainfluenza 3. Signs of exacerbation preceeding symptoms of respiratory infection. Yellow-green coloured sputum from day preceeding SRI onward, lasting for about 1 week. Sputum gram stain positive. Spontaneous return to condition comparable to pre-infection state within 1 week.



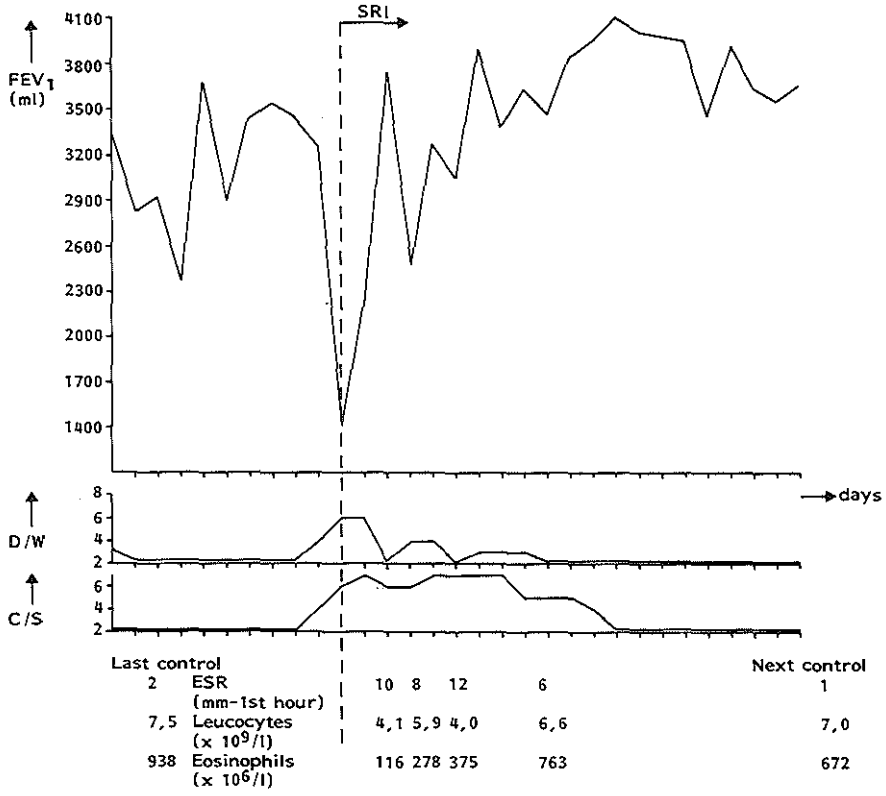

Case report 11

B63 Male 36 yrs Group B-II

SRI symptoms: Malaise, running nose, sneezing, sore throat, dry coughing, headache, myalgia, chest pain, itchy eyes. Maximum rectal body temperature: 38.1° C on 1st day of SRI.

Serology: Rise in CF titre to parainfluenza 3 from <7 to 20.

Prolonged bronchial obstructive exacerbation in the course of SRI by parainfluenza 3. No spontaneous improvement. Return to pre-infection level after administration of high dosages of corticosteroids, on 12th day following begin of SRI (*). Change in sputum colour into yellow-greenish from 2nd day of SRI onward. Sputum gram stain positive. Antibiotics administered for period of 10 days.



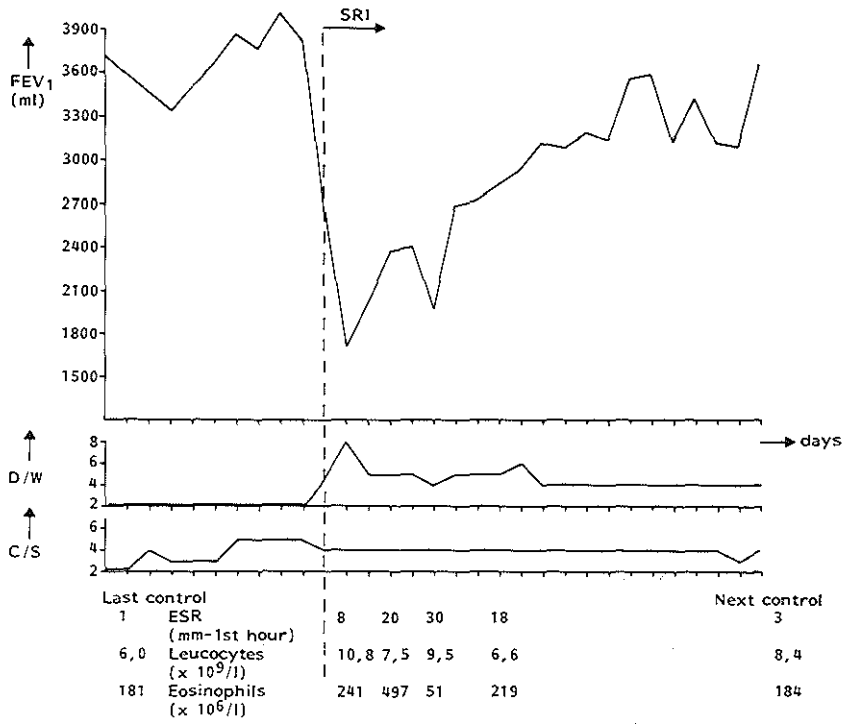

Case report 12

B41 Male 24 yrs Group B-I

SRI-symptoms: Malaise, stuffed nose, sneezing, headache, myalgia, itchy eyes, vomiting. Maximum rectal body temperature: 39.2° C on 1st day of SRI.

Serology: Rise in CF titre to influenza A from <7 to 80.

Short-lasting, but marked increase in bronchial obstruction, related to influenza A infection. Discolouration of sputum from 5th day of SRI onward, lasting 4 days. Sputum gram stain positive. Spontaneous disappearance of productive coughing associated with spontaneous improvement of $FEV_1$ to levels higher than those before SRI.



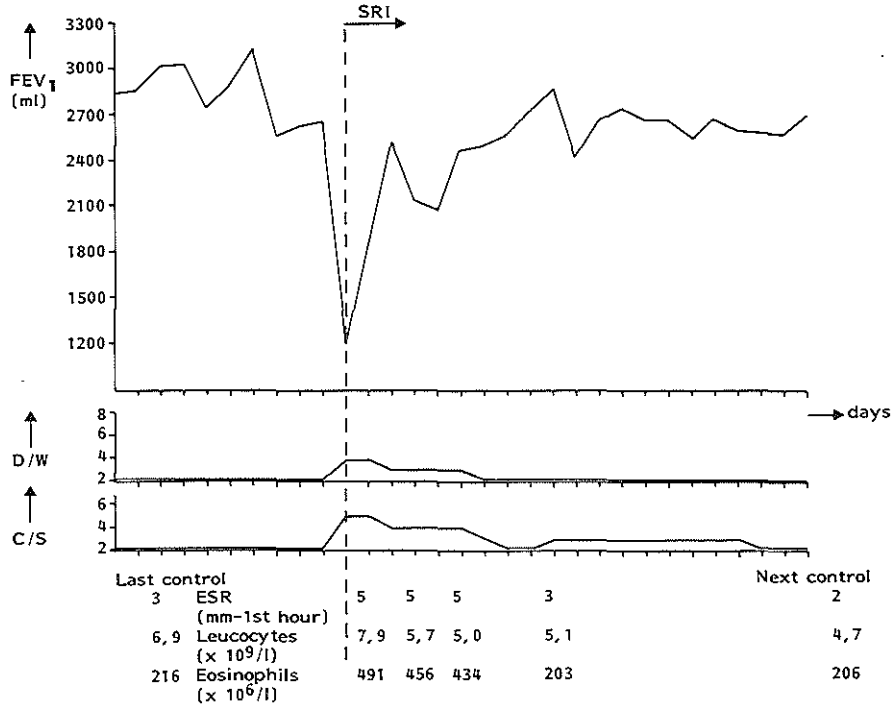

Case report 13

B23 Female 20 yrs Group B-I

SRI symptoms: Malaise, sore throat, running nose, dry coughing, sneezing, hoarseness, chest pain. Mamixum rectal body temperature: 38.5° C on 1st day of SRI.

Serology: Negative.

Marked asthmatic reaction with $FEV_1$ decreasing by more than 50% of baseline value in the course of SRI without positive serology. Spontaneous return to pre-infection $FEV_1$ within 2 weeks. No sputum expectorated during SRI in patient normally coughing up moderate amounts of sputum.



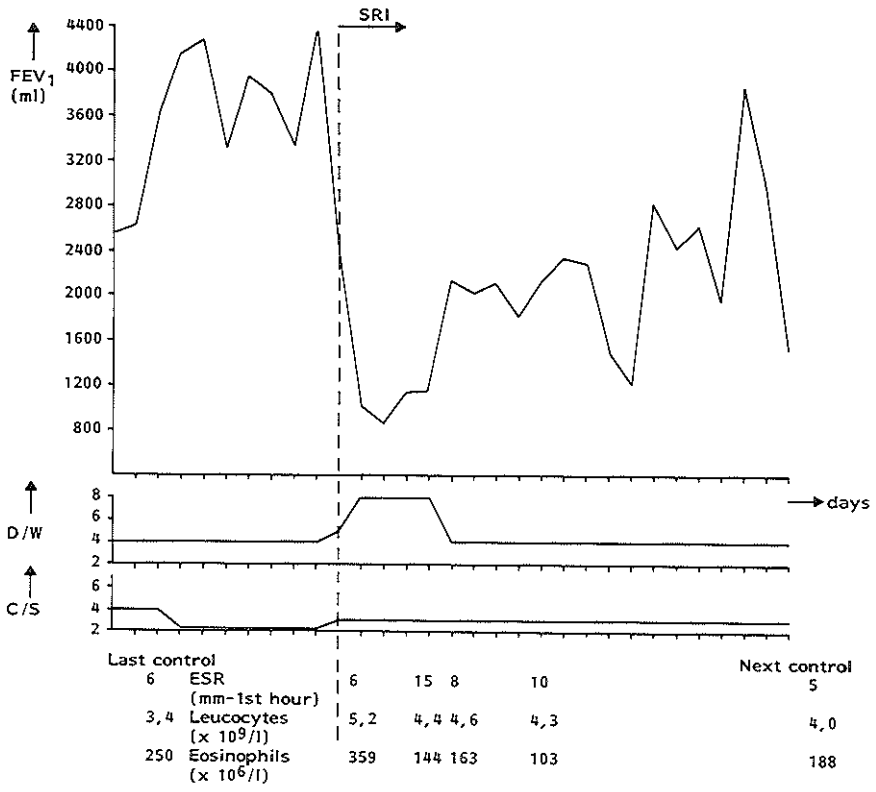

Case report 14

B24 Male 14 yrs Group B-I

SRI symptoms: Malaise, sore throat, running nose, sneezing, headache, chest pain. Maximum rectal body temperature: 39.1° C on 2nd day of SRI.

Serology: Negative.

Short-lasting, but distinct increase of bronchial obstruction in SRI of unknown origin. Expectoration of white coloured sputum starting on 1st day of SRI, lasting for 1 week. No additional medication administered.



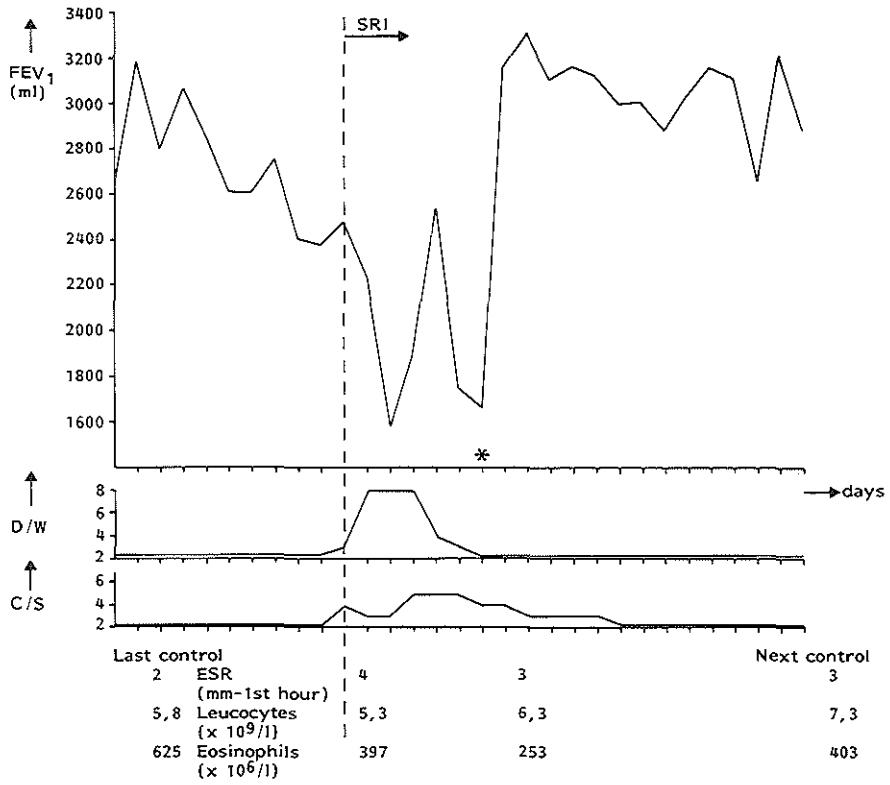

Case report 15

B36 Female 19 yrs Group B-I

SRI symptoms: Malaise, running and stuffed nose, sneezing, sore throat, sore ears, dry coughing. Maximum rectal body temperature: 38.1° C on 1st-3rd day of SRI.

Serology: Negative.

Severe prolonged asthmatic exacerbation during SRI of unknown, possibly common cold-viral origin. Bad control of bronchial obstruction for a period of several weeks with large fluctuations of $FEV_1$. After 4 weeks occurrence of bacterial sputum infection, after which good management of asthma was re-established without problems. Corticosteroid treatment was refused by patient. Additional bronchodilating agents administered on first 2 days of SRI only.



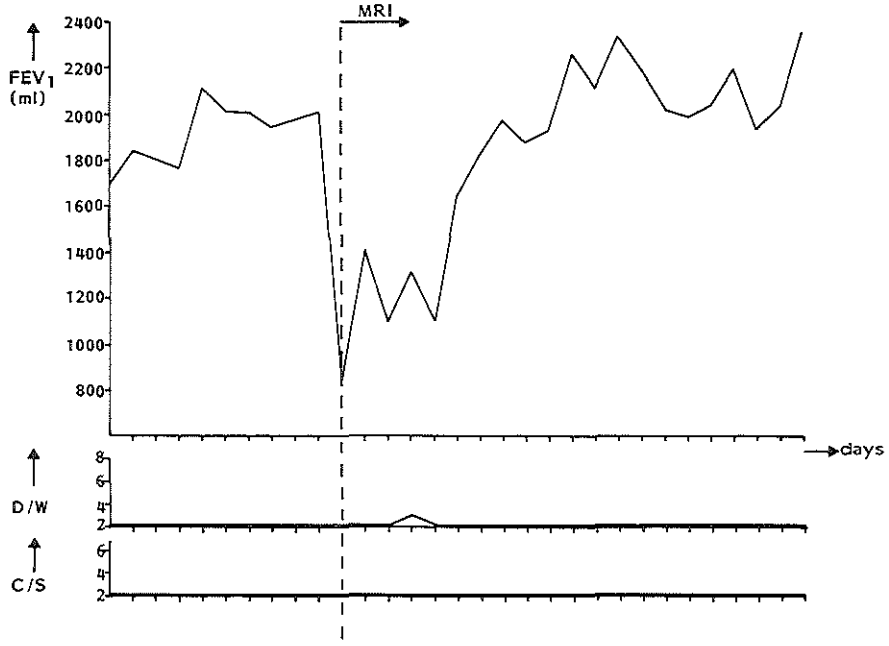

Case report 16

B9 Female 20 yrs Group B-I

SRI symptoms: Malaise, sore throat, running nose, sneezing, dry coughing. Maximum rectal body temperature: 38.5° on 3rd-4th day of SRI.

Serology: Negative.

Period of increased bronchial obstruction in SRI of unknown origin, followed by bacterial sputum infection on 7th day of SRI (*), associated with spontaneous return of $FEV_1$ to pre-infection levels. Bacterial infection, indicated by positive gram stain, was confirmed by culture methods, yielding growth of *Streptococcus pneumoniae*. Antibiotics administered. X-thorax unchanged.

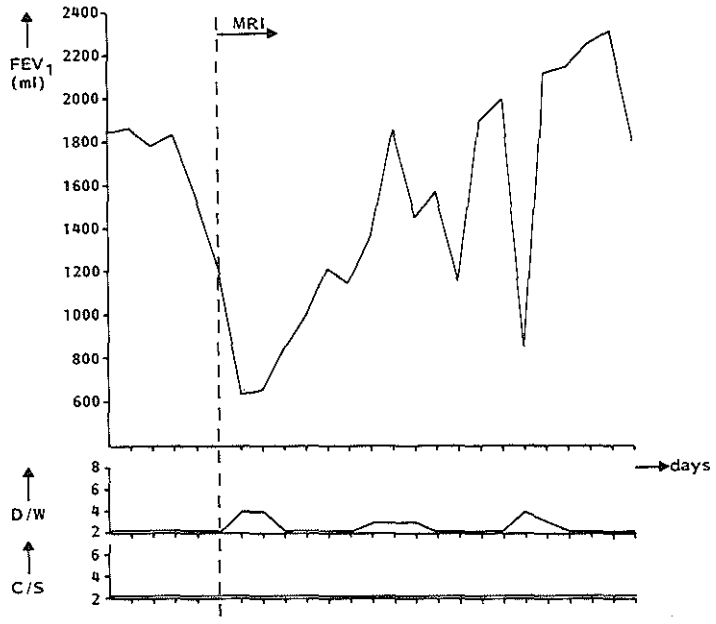



Case report 17

B15 Male 15 yrs Group B-I

MRI symptoms: Sore throat, running nose, both of mild character. Running nose for <36 hrs.
Serology: Rise in CF titre to influenza A from 12 to 80.

Distinct fall in $FEV_1$ in the course of influenza A infection with mild upper respiratory symptoms (MRI). No significant changes in scores for dyspnoea/wheezing and cough/sputum. Spontaneous return to pre-infection level of $FEV_1$ within 1 week.

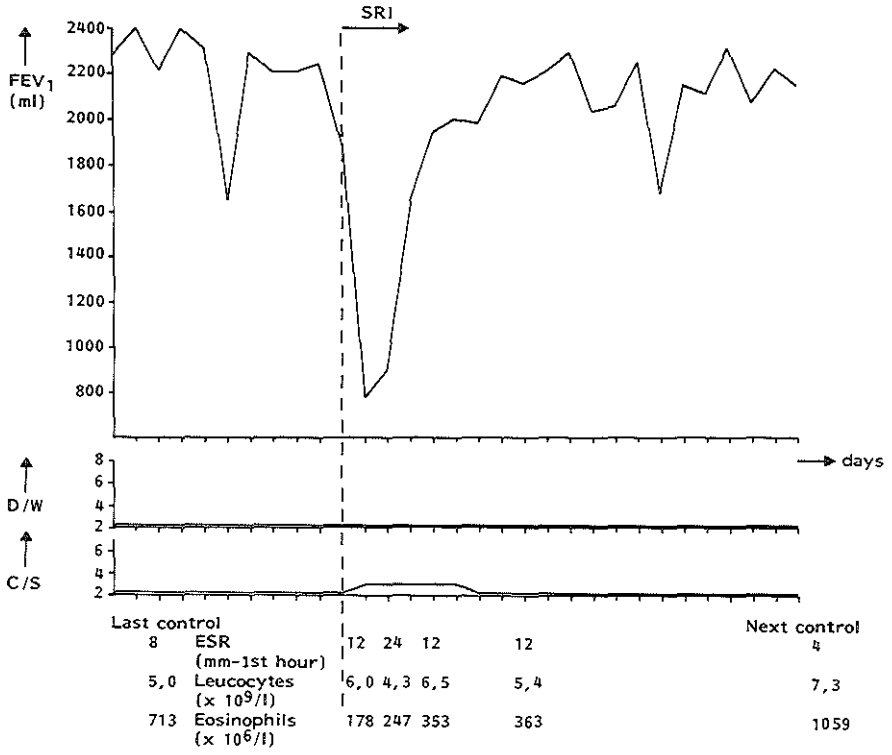



Case report 18

B72 Female 18 yrs Group B-I

MRI symptoms: Malaise
Serology: Rise in CF titre to parainfluenza from <7 to 96.

Fall in $FEV_1$ associated with parainfluenza 3 infection without upper respiratory symptoms. Short-lasting increase in dyspnoea/wheezing, no change in cough/sputum. $FEV_1$ returned to pre-infection levels within 10 days, but large variations in $FEV_1$ were present for a period of about 4 weeks.



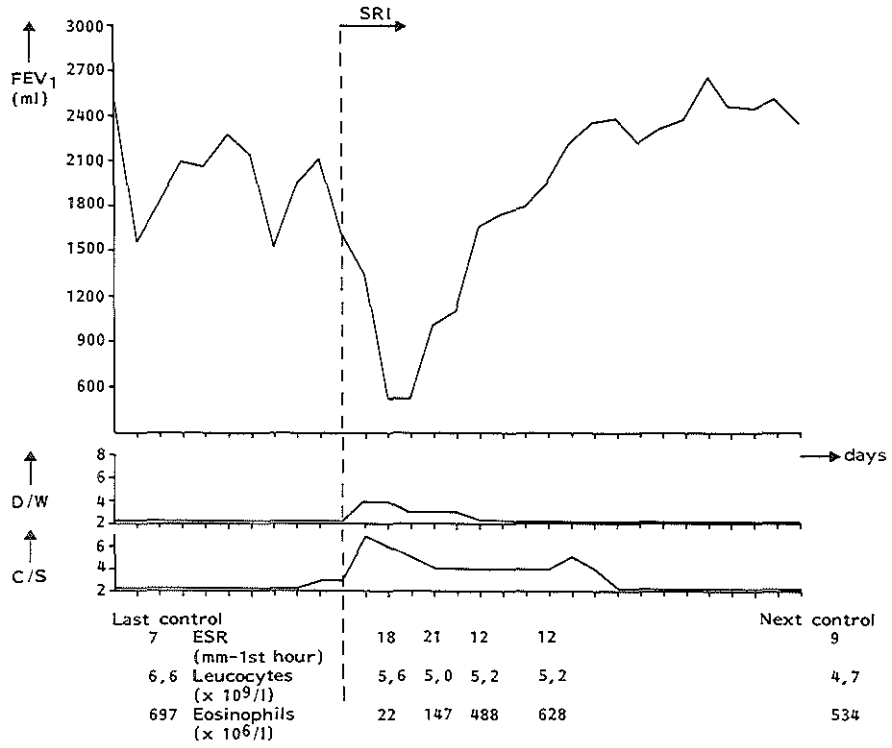

Case report 19

C8 Male 9 yrs Group C-I

SRI symptoms: Malaise, running nose, sneezing, headache, itchy eyes. Maximum rectal body temperature 38.2° C on 1st day of SRI.

Serology: Rise in HI titre to influenza B/Hong Kong/8/72 from 60 to 480.

Short-lasting but serious fall in $FEV_1$ in SRI by influenza B. Despite decrease of $FEV_1$ of over 50% of baseline value, increase in dyspnoea/wheezing was denied. Spontaneous return to baseline $FEV_1$ values. No expectoration of sputum.



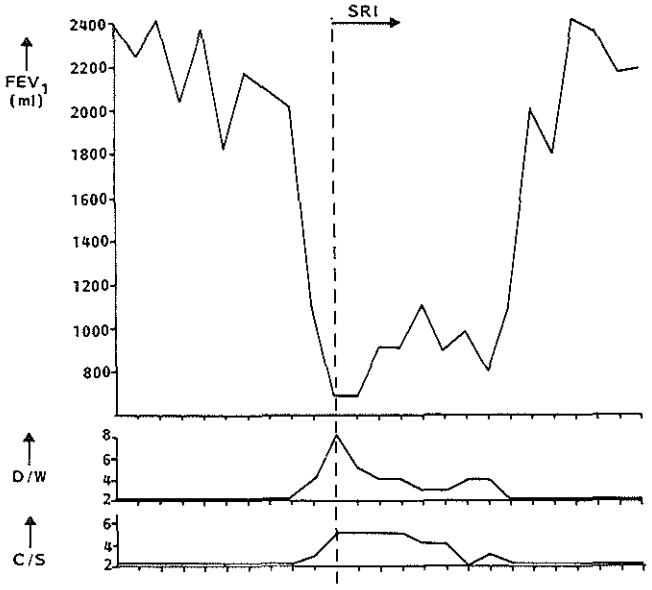

Case report 20

C14 Male 13 yrs Group C-I

SRI symptoms: Malaise, running and stuffed nose, sore throat, dry coughing, headache, myalgia. Maximum rectal body temperature: 40° C on 2nd day of SRI.

Serology: Rise in HI titre to influenza A/Texas/1/77(H3N2) from 30 to 1100.

Serious asthmatic exacerbation with $FEV_1$ decreasing to 520 ml in SRI by influenza A. Spontaneous return to baseline value within 1 week. Yellow-green coloured sputum expectorated from 3rd to 6th day of SRI. Sputum gram stain positive. No additional medication. X-thorax unchanged.

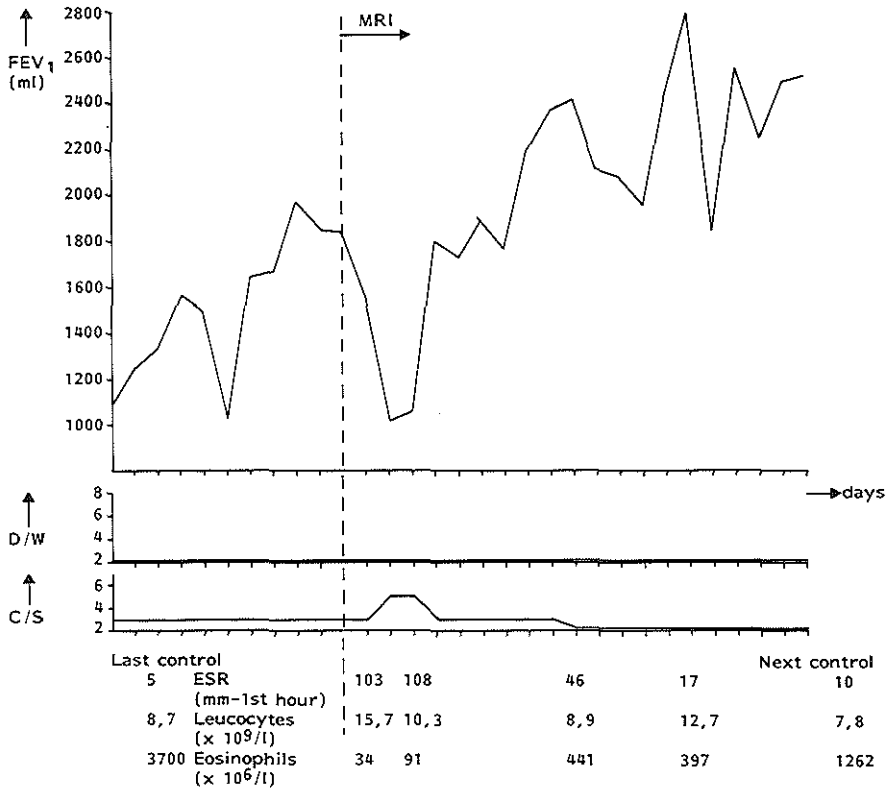



Case report 21

C14 Male 13 yrs Group C-I

SRI symptoms: Running nose, sore throat. Maximum rectal body temperature 38.7° C on 2nd day of SRI.
Serology: Rise in CF titre to *Mycoplasma pneumoniae* from <7 to 56.

Relatively prolonged, severe asthmatic reaction to *Mycoplasma pneumoniae* infection with few upper respiratory symptoms. SRI was preceeded by drop in $FEV_1$. White sputum expectorated for first 6 days of SRI. Nevertheless, antibiotics were administered. Unfortunately, laboratory data were not collected according to programme and chest röntgenograms were not made.



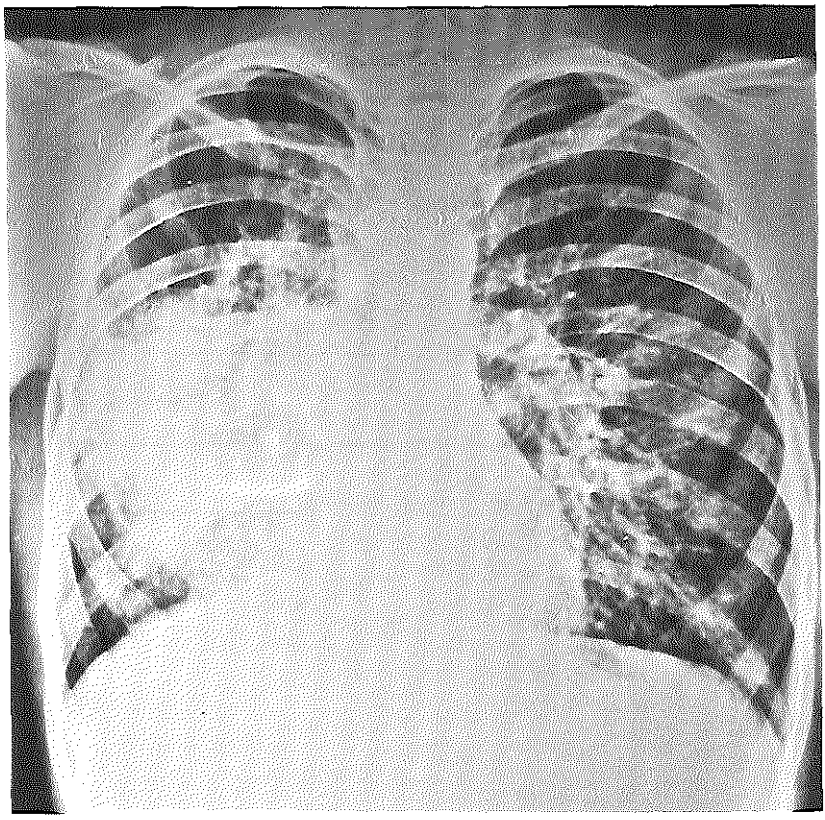

Case report 22

C17 Male 9 yrs Group C-I

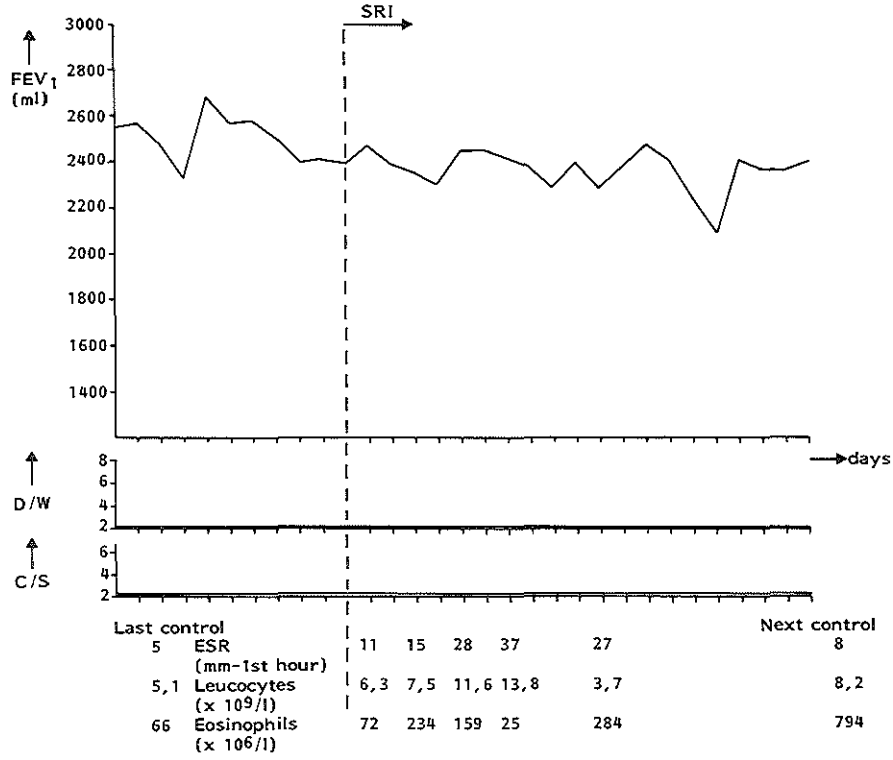
MRI symptoms: Chest pain. Maximum rectal body temperature: 40° C on 3rd day of MRI.

Serology: Rise in CF titre to influenza A from 8 to 32.

Severe bronchopneumonia in the course of influenza A infection without upper respiratory symptoms in boy with extremely difficultly managable bronchial asthma and severe anatomic deformations in right middle and upper lobe. Increased bronchial obstruction associated with expectoration of purulent sputum -gram stain positive, *Streptococcus pneumoniae* in culture- on 3rd and 4th day of MRI. Admission to intensive care unit, where treatment with corticosteroids and antibiotics was begun. X-thorax see page 220.



X-thorax patient C17 (case report 22). Diffuse, dense infiltrate in right middle and lower lung fields. Patchy infiltrate in left costo-diaphragmatic angle.



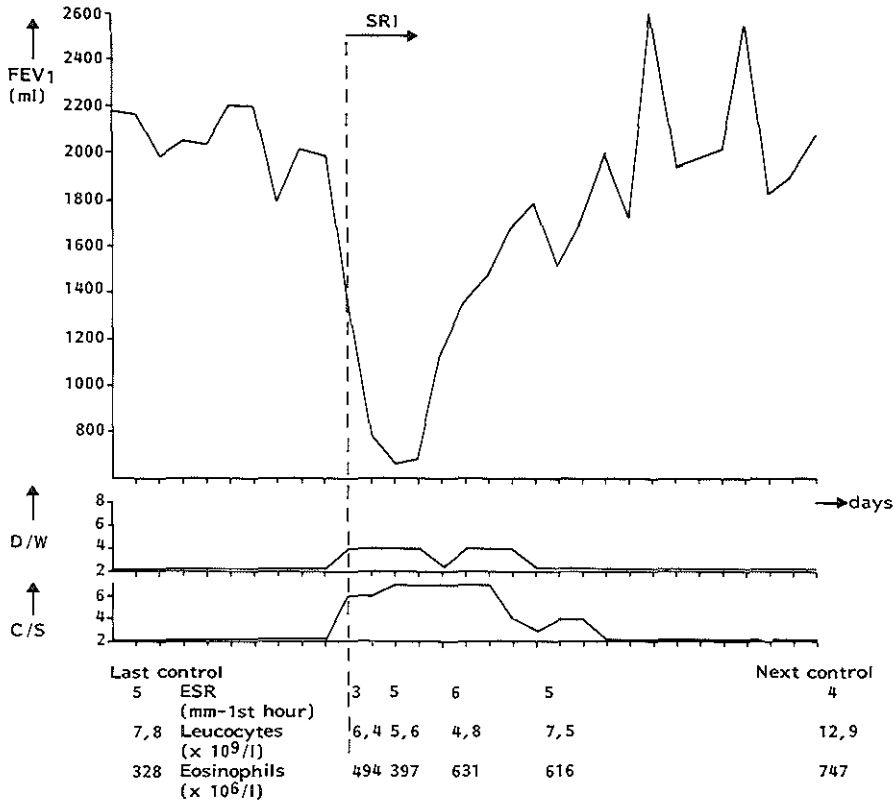

Case report 23

C4 Male 10 yrs Group C-I

SRI symptoms: Malaise, sore throat, sore ears, dry coughing, headache. Maximum rectal body temperature: 37.9° C on 1st day of SRI.

Serology: Negative.

Good control of bronchial obstruction in SRI, caused by β-haemolytic group A streptococci. No changes in scores for dyspnoea/wheezing and cough/sputum and very consistent values of $FEV_1$.

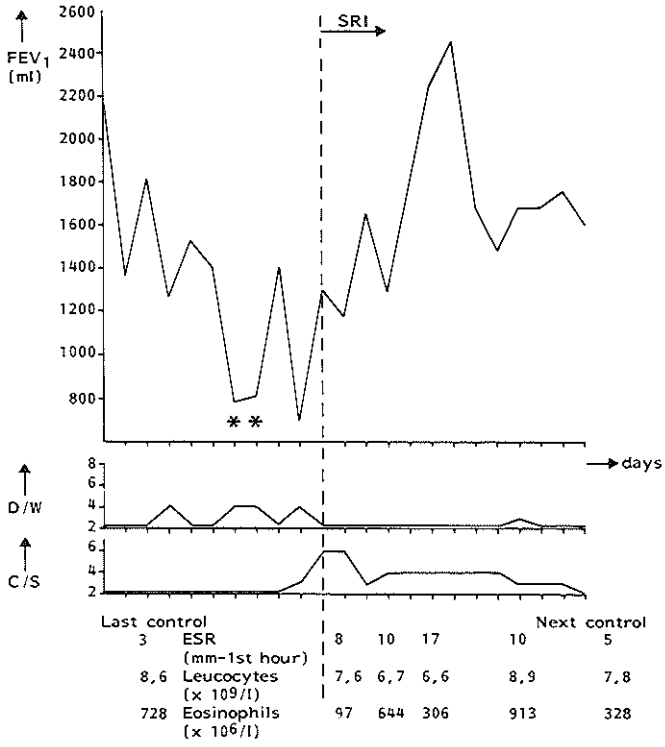



Case report 24

CII Male 12 yrs Group C-II

SRI symptoms: Running and stuffed nose, dry coughing, malaise. Maximum rectal body temperature: 37.7° C on 2nd day of SRI.
Serology: Negative.

Serious bronchial obstructive reaction associated with SRI of unknown, possibly common cold-viral origin. Spontaneous return to pre-infection $FEV_1$ values within 1 week. Purulent sputum from 2nd day of SRI onward, lasting for 6 days. Sputum gram stain positive. Antibiotics were administered. X-thorax unchanged.



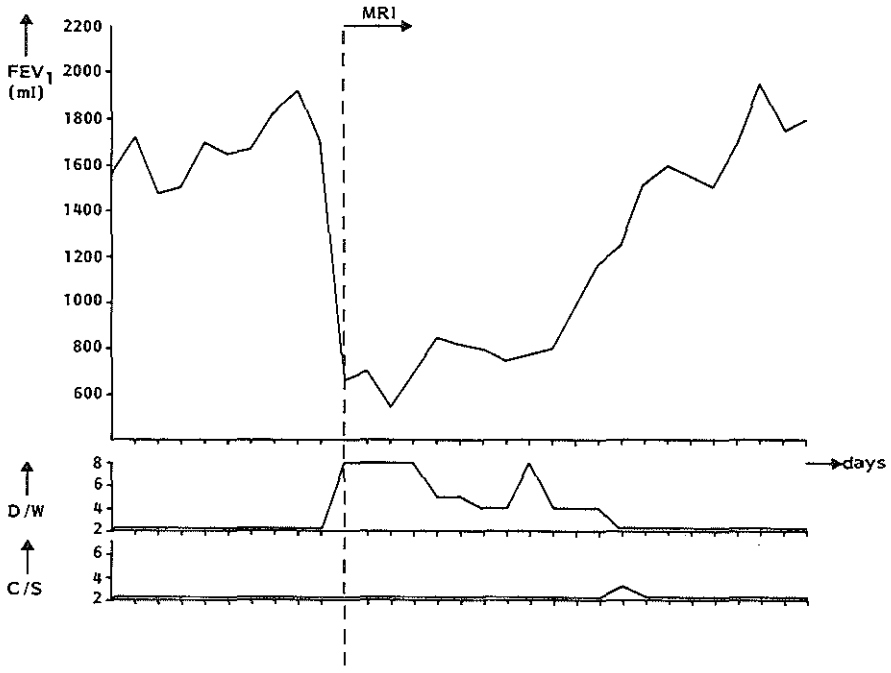

Case report 25

C11 Male 12 yrs Group C-II

SRI symptoms: Malaise, stuffed nose, sore throat, hoarseness, headache, myalgia, chest pain, itchy eyes. Maximum rectal body temperature: 39.3° C on 1st day of SRI.

Serology: Negative.

Poor control of asthma with exacerbation induced by dog allergen provocation (*), followed be bacterial sputum infection -gram stain positive, *Streptococcus pneumoniae* and *Haemophilus influenza* in culture- associated with SRI. Spontaneous improvement of $FEV_1$, however, with large variations. Antibiotics installed. X-thorax unchanged.

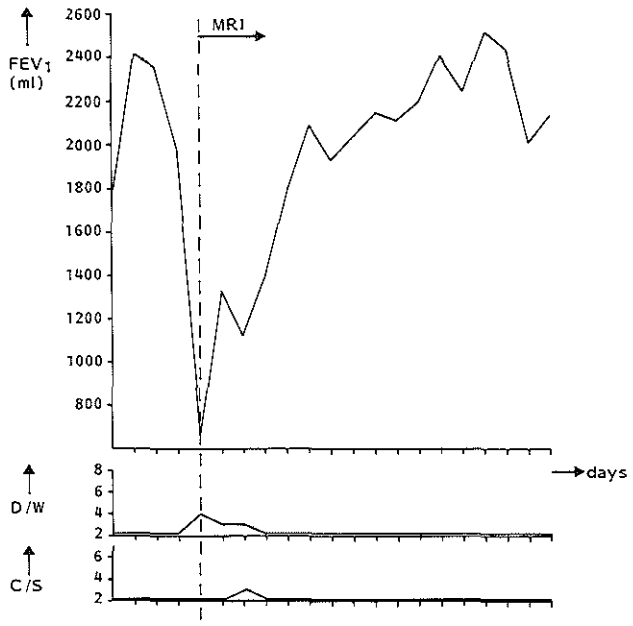



Case report 26

C21 Male 14 yrs Group C-I

MRI symptoms: Slight malaise
Serology: Rise in CF titre to influenza A from <7 to 48.

Serious bronchial obstructive reaction with prolonged duration in influenza A infection without any other respiratory symptoms. Spontaneous recovery within 2 weeks. No expectoration of sputum.

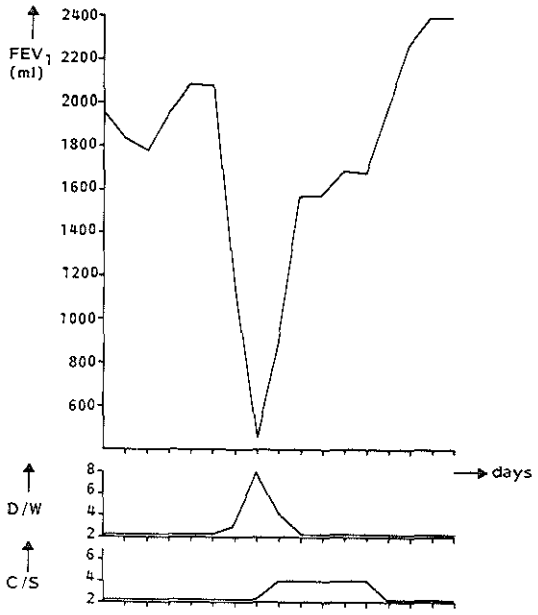



Case report 27

C14 Male 13 yrs Group C-I

MRI symptoms: Malaise
Serology: Rise in CF titre to adenovirus from 7 to 40.

Short-lasting, but distinct bronchial obstructive reaction in patient with MRI by adenovirus. No other respiratory symptoms. Spontaneous return to pre-infection $FEV_1$ values.

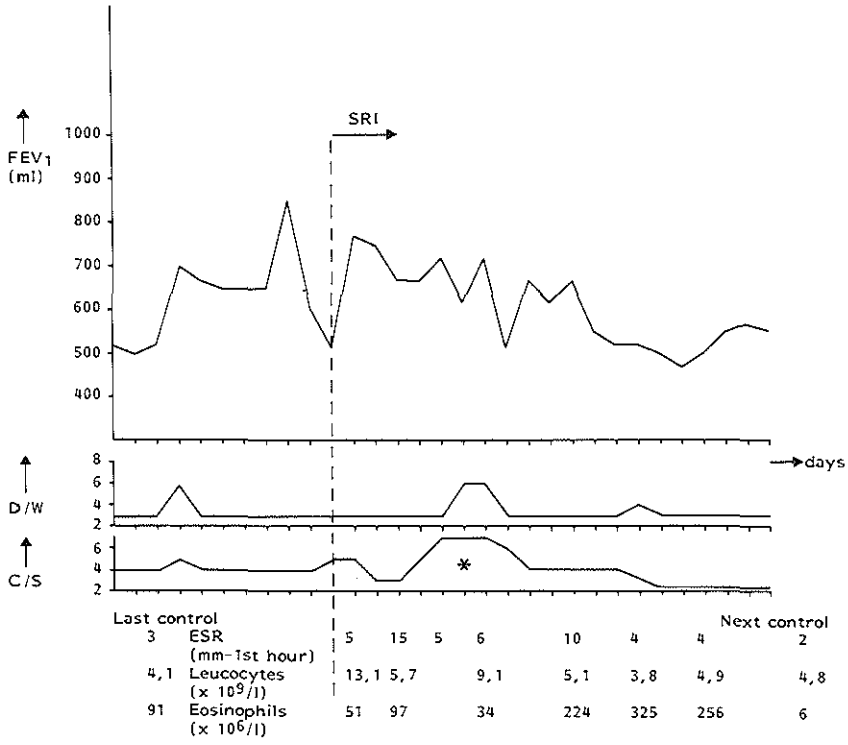



Case report 28

C14 Male 13 yrs Group C-I

Symptoms of respiratory infection : None
Serology : Rise in HI titre to influenza A/Victoria/3/75 (H3N2) virus from 135 to 1100.

Short-lasting severe bronchial obstructive exacerbation in a boy whose asthma had been well-controlled before. Significant rise in titre to influenza A at the same time, possibly related to increase of asthmatic signs and symptoms.




Case report 29

A119 Male 65 yrs Group A-III

SRI symptoms: Malaise, dry coughing, running nose, headache, myalgia, chest pain, vomiting. Maximum rectal body temperature: 39.2° C on 2nd day of SRI.

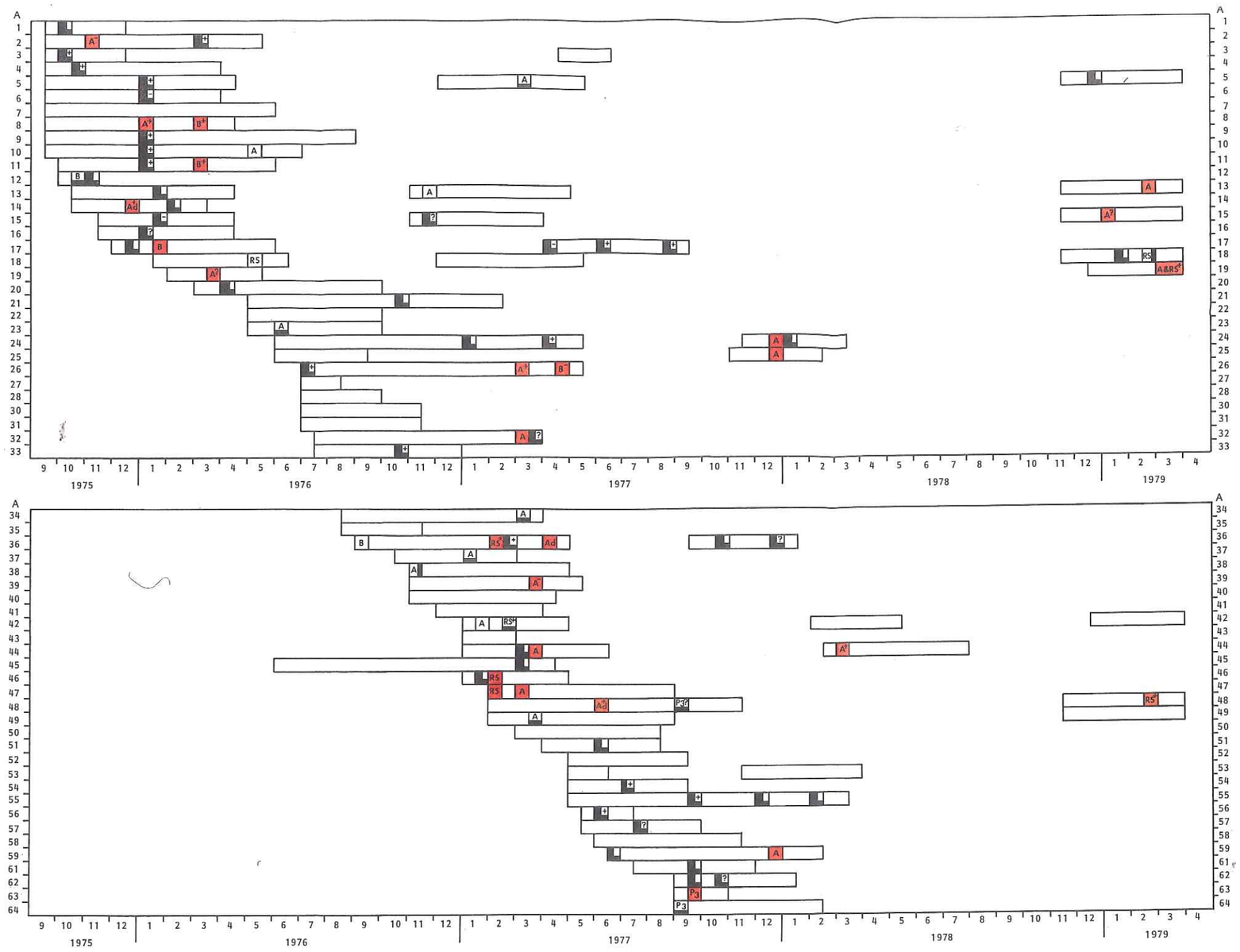
Serology: Rise in CF titre to parainfluenza 3 from <7 to 80.

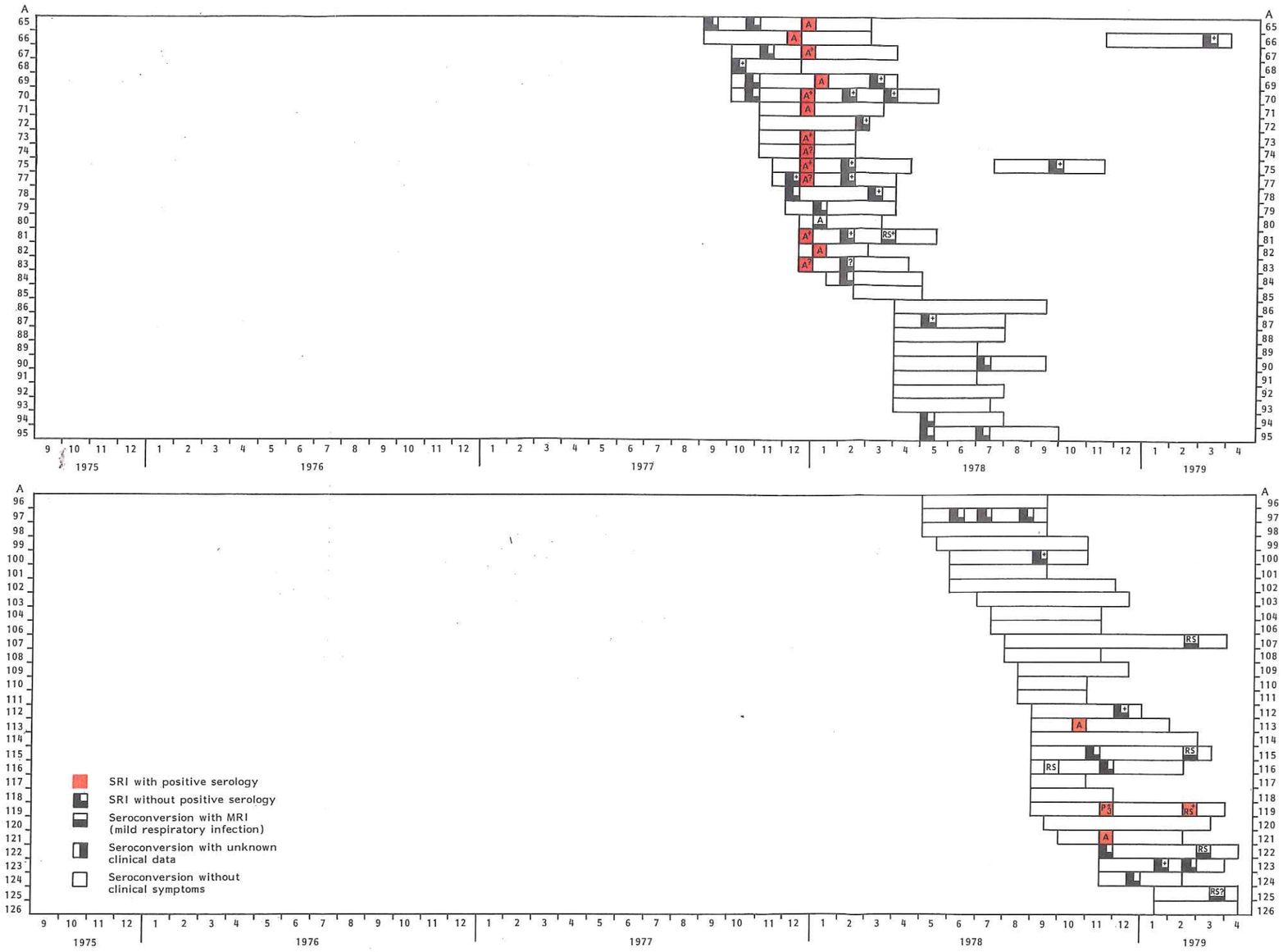
SRI by parainfluenza 3 in patient with severe chronic airflow obstruction. No significant changes in $FEV_1$. Increased dyspnoea/wheezing and cough/sputum after 1 week with discolouration of sputum on day 7 (*). Sputum gram stain positive with *Streptococcus pneumoniae* and *Haemophilus influenzae* in culture. Antibiotic treatment was given. X-thorax unchanged.

# APPENDIX II

3-3 Periods of SRI, seroconversions without SRI, and results of
n gram stain investigations in individual patients of group A.

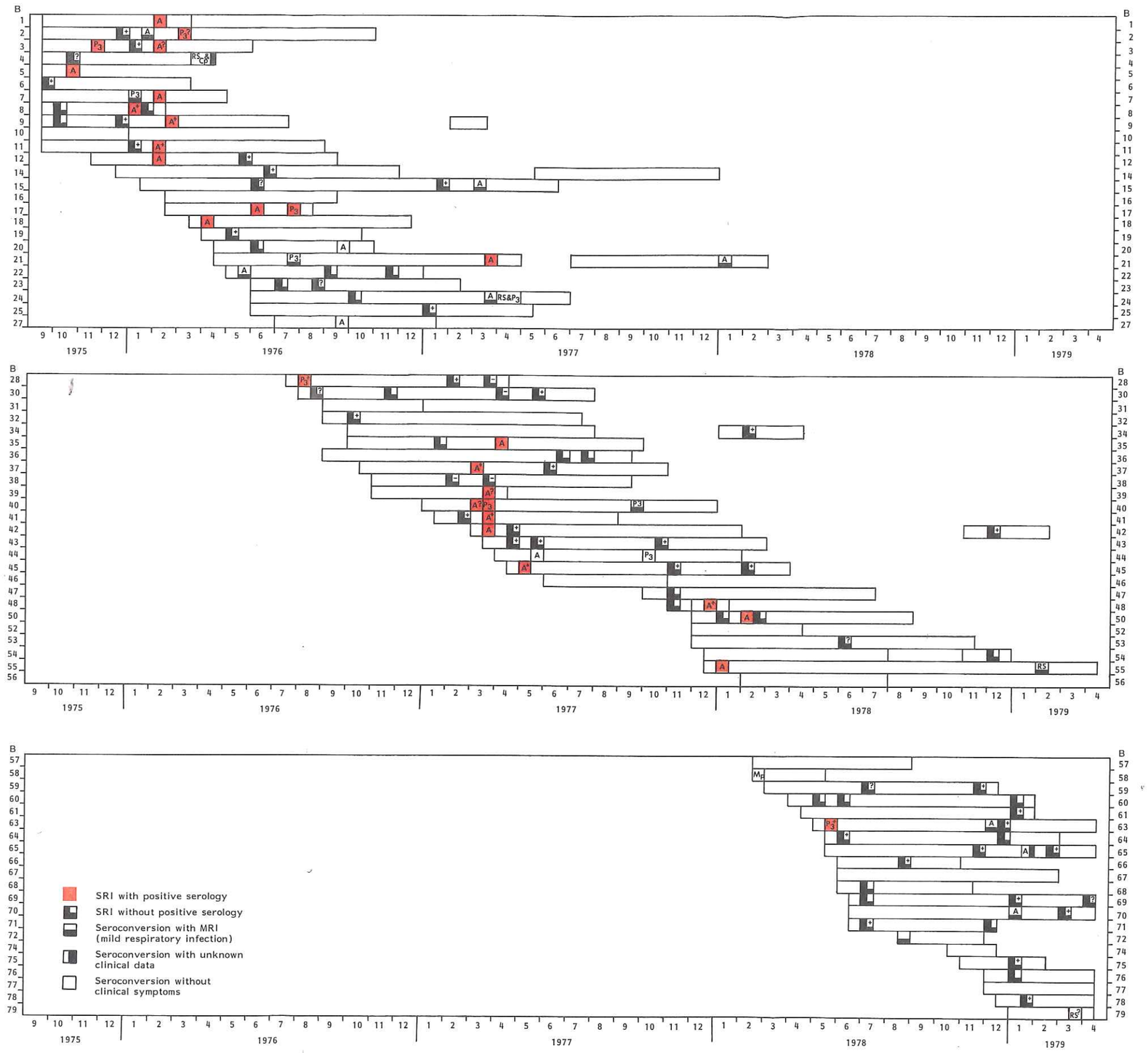
A = Influenza A virus
B = Influenza B virus
$P_3$= Parainfluenza 3 virus
Ad= Adenovirus
RS= Respiratory syncytial virus
+ = positive sputum gram stain
− = negative sputum gram stain
? = yellow/green sputum, no gram stain made




SRI with positive serology
SRI without positive serology
Seroconversion with MRI (mild respiratory infection)
Seroconversion with unknown clinical data
Seroconversion without clinical symptoms
1975
1976
1977
1978
1979

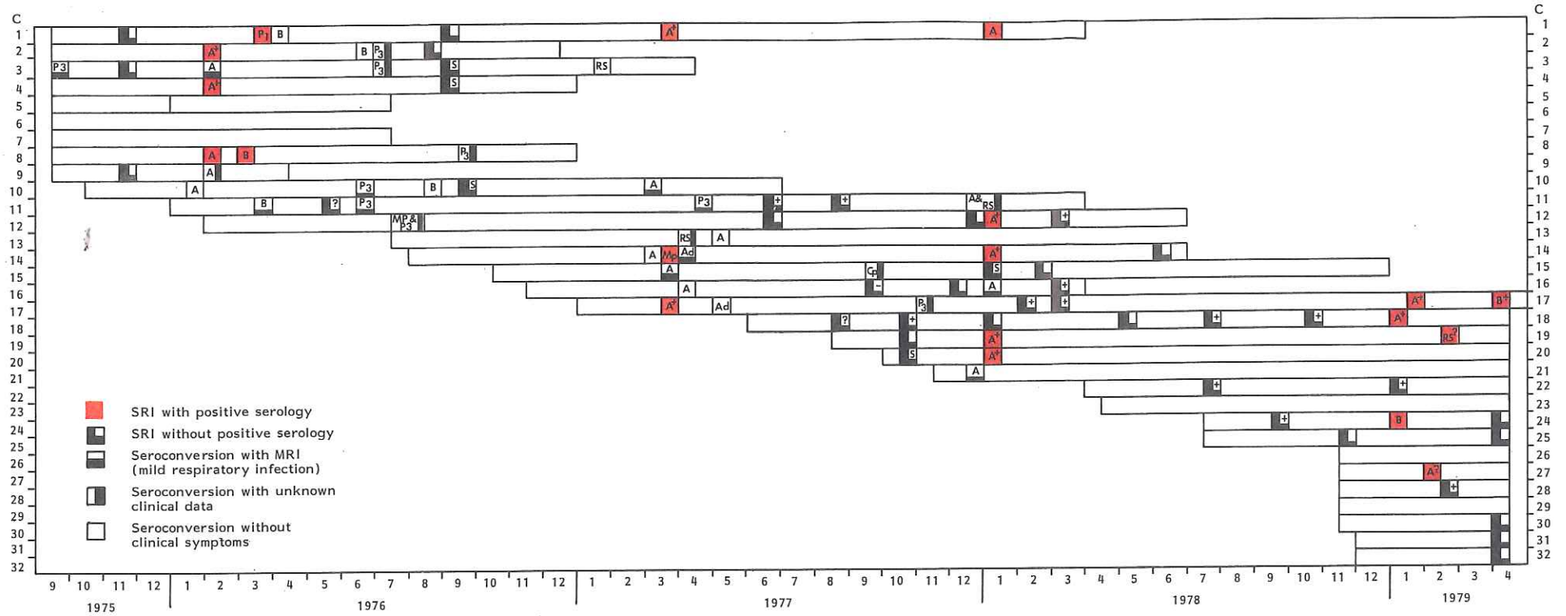
; Periods of SRI, seroconversions without SRI, and results of
;ram stain investigations in individual patients of group B

A = Influenza A virus
$P_3$= Parainfluenza 3 virus
RS= Respiratory syncytial virus
Mp= *Mycoplasma pneumoniae*
Cp= *Chlamydia psittaci*
+ = positive sputum gram stain
- = negative sputum gram stain
? = yellow/green sputum, no gram stain made



) Periods of SRI, seroconversions without SRI, and results of ;ram stain investigations in individual patients of group C.

A = Influenza A virus
B = Influenza B virus
$P_1$= Parainfluenza 1 virus
$P_3$= Parainfluenza 3 virus
Ad= Adenovirus
RS= Respiratory syncytial virus
Mp= *Mycoplasma pneumoniae*
Cp= *Chlamydia psittaci*
S = β haemolytic group A streptococci
+ = positive sputum gram stain
- = negative sputum gram stain
? = yellow/green sputum, no gram stain made



*Dag, het is leuk geweest.*
*Fijn voor een moment in mijn leven*
*dat reeds achter de rug is.*
*Hallo en tot ziens.*
*Bedankt.*

*Fred van Esch*