

# Quality of life, stress and cortisol and acute bronchiolitis in infancy and early development of atopic disease

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Lung and Environment

2020



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*Series of dissertations submitted to the  
Faculty of Medicine, University of Oslo*

ISBN 978-82-8377-625-6

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Cover: Hanne Baadsgaard Utigard.  
Photo cover: Ine Eriksen, UiO  
Print production: Reprosentralen, University of Oslo.

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## ACKNOWLEDGMENTS

For this project, participation of more than 600 children and their parents and innumerable health care professionals including the Bronchiolitis Study Group of the Oslo Research group on Asthma and Allergy in Children, the Lung and Environment (ORAACLE) was indispensable, and I will warmly thank all of them. I am especially thankful to my main supervisor, Professor Karin C. L. Carlsen, paediatrician and leader of ORAACLE, for being very enthusiastic, informative, helpful, supporting and to the point through all stages from the early conception through the final writing of the articles and thesis. In addition, Professor Kai-Håkon Carlsen has been very helpful, supporting and enduring. Egil Bakkeheim has been a helpful supervisor, especially within the area of cortisol and asthma. I think in grief, but gratitude of my statistician, Petter Mowinckel, who recently passed away, for being so supporting, helpful, cheerful and available at different times of the day. I will also like to thank the principal investigator of the Bronchiolitis study, Håvard Ove Skjerven, for being supportive and focused, and to co-investigator and dermatologist, Teresa Løvold Berents for all help also including evaluation of atopic dermatitis, and to co-investigators Jon Olav Hunderi, Bente Kvenshagen and Karen Eline Stensby Bains for important help and support, and to study nurses Solveig Knutsen, Live Nordhagen, Liv Julie Sjørdal and Anne Birgitte Magneshaugen for all their patience and practical help. I am also grateful for help from the IT-supporting staff of Innlandet Hospital. Thanks to Professor Johan Alm and biochemical engineer Ann-Christine Sjöbeck at the Karolinska Institute, for analyses of the salivary samples. I would like to thank my son, Sigmund Rolfsjord, master and researcher of information technology, for helping me setting up syntaxes in SPSS, especially in the early stages, and to lecturers and responsible staff for the PhD programme at the University of Oslo for indispensable information and teaching, especially within the fields of research methods and statistics. I am also grateful to the Norwegian state railway company Vy for providing comfortable wagons, seats and facilities for working with a laptop during countless trips to

Oslo. Finally, I will like to thank my wife, Randi, for her everlasting endurance and for encouraging me to complete this thesis.



## SUMMARY OF THE THESIS

### Introduction:

Reduced health related quality of life (QoL) is reported in children with asthma, atopic dermatitis and viral lower respiratory infections. Acute viral bronchiolitis in infancy is an asthma risk factor. Reduced QoL may be associated with physical and psychological stress. Acute stress has been associated with higher morning salivary cortisol, whereas chronic stress in children with asthma and atopic dermatitis may influence the hypothalamus-pituitary-adrenal (HPA)-axis, resulting in a reduced cortisol response to acute stress. Possibly, this influence on the HPA-axis can be found also before asthma has fully evolved.

### Objectives

Our main objective was to explore how QoL and salivary cortisol as a surrogate marker for stress may contribute to the development of asthma and atopic diseases in young children with or without acute bronchiolitis in infancy.

### Specific aims

Our specific aims were as follows:

1. To investigate if acute bronchiolitis in infancy and severity of the disease are associated with reduced QoL in young infants (papers 1, 2 and 4).
2. Using salivary cortisol as a marker of stress, to determine if stress is associated with acute bronchiolitis and/or development of asthma or other atopic diseases (papers 3 and 4).
3. To determine if QoL is associated with asthma risk factors, asthma and/or other atopic diseases in young children with or without acute bronchiolitis in infancy (papers 1 and 4).
4. To determine if there is a link between QoL, salivary morning cortisol and atopic diseases, and if such a link may be modified by acute moderate to severe bronchiolitis in infancy (paper 4).

## Study design, subjects and methods

To answer the aims we used data from the Bronchiolitis ALL-study, SE-Norway, comprising 404 children <12 months of age included in a randomised placebo-controlled trial of treatment of acute moderate to severe bronchiolitis in infancy as well as 240 infants of similar age recruited as controls for the prospective arm of the study, from the general population.

Morning salivary cortisol was collected on awakening the first morning after inclusion in the study and at the two-year follow-up investigation. Standardised Infant Toddler Quality of Life Questionnaire (ITQOL-97™) 13-domain questionnaires were completed by caregivers at home nine months after inclusion and prior to the two-year follow-up investigation.

Recurrent bronchial obstruction (rBO) used as a proxy for asthma was defined as at least three parent reported episodes of wheeze by two years of age.

## Results

At enrolment, salivary cortisol was available from 383 infants, QoL was assessed in 415 at mean age 14 months, while at two years, salivary cortisol was available from 379 children, QoL in 453, and both in 358 children, of whom 203 were hospitalised for acute bronchiolitis at inclusion and 155 were controls. The Overall health and General health domains of QoL were significantly lower among children who had been hospitalised with acute bronchiolitis in infancy compared to controls at both assessments, at mean ages of 14 and 24 months, respectively. At a mean age of 14 months, longer hospital stay was associated with lower QoL in five domains, and supportive treatment (nasogastric tube feeding, oxygen supply or ventilatory support) with lower QoL in four domains.

Morning salivary cortisol was significantly higher in infants hospitalised for acute bronchiolitis than in controls at enrolment. The two groups had similar cortisol levels at two years of age, although higher in girls, whereas morning salivary cortisol was significantly reduced in children with rBO.

Asthma risk factors, especially atopic dermatitis, were significantly associated with lower QoL nine months after enrolment. More than three asthma risk factors among children who were hospitalised with acute bronchiolitis had lower scores in four domains and controls in three domains. At two years of age, reduced QoL was significantly associated with developing rBO, and to a lesser extent, but with at least five per cent with atopic dermatitis for three domains, while significantly associated with allergic sensitisation only among controls.

At two years of age, lower cortisol levels were associated with reduced QoL in eight domains in the bronchiolitis group, but with General health only among controls. Adjusting for rBO reduced the magnitude of the associations, but still rendering the associations statistically significant in eight domains. However, the impact of atopic dermatitis on the associations was less clear.

In the bronchiolitis group, most QoL domains at the first survey was significantly associated with morning salivary cortisol at two years of age.

## Discussion

Our findings of reduced QoL after acute bronchiolitis is consistent with a smaller study, but the impact of common asthma risk factors on QoL in a group of children hospitalised for acute bronchiolitis in infancy compared to a control group was novel. Nine months after acute bronchiolitis and at two years of age, QoL in the bronchiolitis group was significantly reduced compared with the control group only for the domains Overall health and General health, and increased for the domain Change in health, as previously seen in chronic disease. Atopic dermatitis at inclusion had a stronger association with QoL nine months later than atopic dermatitis at two years of age with QoL at two years of age, possibly attributed to poorer prognosis in early onset atopic dermatitis.

The higher morning salivary cortisol in infants at hospitalisation for acute bronchiolitis, may be explained by acute stress in this early stage of development.

Our findings support that chronic stress, indicated by lower morning salivary cortisol associated with low QoL in young children with an increased risk for asthma, e.g. after acute bronchiolitis in infancy and in children with rBO, may contribute to the development of asthma. This was supported by rBO reducing the association between cortisol and QoL at two years of age and QoL nine months after inclusion and cortisol at two years of age. Thus, rBO appears linked to the association between QoL and cortisol, indicating a role of chronic stress in the early development of asthma.

## Conclusion

Moderate to severe acute bronchiolitis in infancy, the severity of the acute bronchiolitis and common asthma risk factors were negatively associated with QoL nine months later.

Hospitalisation for acute bronchiolitis in infancy was associated with higher concurrent morning salivary cortisol.

Asthma, expressed by rBO as a proxy, and atopic dermatitis were associated with reduced QoL.

In children who were hospitalised for acute bronchiolitis in infancy, low QoL was associated with low morning concurrent salivary cortisol at two years of age. This association could partly be explained by asthma. Low QoL at 14 months was also associated with low cortisol at two years of age in these children.

## LIST OF PAPERS

### **Paper 1**

Children hospitalised with bronchiolitis in the first year of life have a lower quality of life nine months later.

Rolfsjord LB, Skjerven HO, Bakkeheim E, Carlsen KH, Hunderi JO, Kvenshagen BK, Mowinckel P, Lødrup Carlsen KC.

Acta Paediatrica 2015 Jan;104(1):53-8. doi: 10.1111/apa.12792.

### **Paper 2**

The severity of acute bronchiolitis in infants was associated with quality of life nine months later.

Rolfsjord LB, Skjerven HO, Carlsen KH, Mowinckel P, Bains KE, Bakkeheim E, Lødrup Carlsen KC.

Acta Paediatrica 2016 Jul;105(7):834-41. doi: 10.1111/apa.13396.

### **Paper 3**

Morning Salivary Cortisol in Young Children: Reference Values and the Effects of Age, Sex, and Acute Bronchiolitis.

Rolfsjord LB, Bakkeheim E, Berents TL, Alm J, Skjerven HO, Carlsen KH, Mowinckel P, Sjöbeck AC, Carlsen KCL.

Journal of Pediatrics 2017 May;184:193-198.e3. doi: 10.1016/j.jpeds.2017.01.064.

### **Paper 4**

Quality of life, salivary cortisol and atopic diseases in young children.

Rolfsjord LB<sup>1,2,3</sup>, Skjerven HO<sup>2,3</sup>, Bakkeheim E<sup>2</sup>, Berents TL<sup>4</sup>, Carlsen KH<sup>2,3</sup>, Carlsen KCL<sup>2,3</sup>

PLoS One. 2019 Aug 30;14(8):e0214040. doi: 10.1371/journal.pone.0214040. eCollection 2019.

## ABBREVIATIONS

QoL	Health related quality of life
QoL <sub>1</sub>	QoL data from first survey, by ITQOL.
QoL <sub>2</sub>	QoL data from second survey, by ITQOL.
HPA-axis	Hypothalamic-pituitary-adrenal axis
ACTH	Adrenocorticotrophic hormone, corticotrophin
Th1, Th2	T-lymphocyte-helper cell 1, T-lymphocyte-helper cell 2
ITQOL	The Infant Toddler Quality of Life Questionnaire™
SCORAD index	Scoring atopic dermatitis index
LOS	Length of stay intention-to-treat
CI	Confidence interval
RBO	Recurrent bronchial obstruction, at least three wheeze episodes
BO	Bronchial obstruction
RSV	Respiratory syncytial virus

## GENERAL INTRODUCTION

### Atopic disease

Atopic diseases including asthma and atopic dermatitis are potentially serious and widespread in the general population including children. In 2010, a Norwegian study of two-year-old children reported a prevalence of doctor diagnosed asthma of 7 %, atopic dermatitis in 17 %, allergic rhinitis in 3 % and positive skin prick tests in 8 % of randomly selected subjects (1). As early childhood is an important period for the development of atopic disease with possible health consequences for the rest of the subjects' lives (2), increasing the understanding of the factors and mechanisms for developing these diseases in early childhood is important to reduce the burden of these diseases. This thesis explores atopic disease development related to early acute bronchiolitis and asthma risk factors and the potential role of stress using cortisol (3) and health-related quality of life (4) as markers to contribute to a better understanding of some of the mechanisms involved in the development of atopic disease in early childhood (5, 6).

### Concepts of atopic diseases

#### Asthma

Asthma is a chronic inflammatory disease that involves a genetic disposition and environmental factors with a variation in prevalence of severe asthma symptoms among 6-7 year old children between 0% in poor countries to 20 % in high income countries.(7) .

The Global Initiative for Asthma (GINA) 2018 defines asthma as “.... a heterogeneous disease, usually characterised by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation” (8). As this definition is difficult to apply in young children, the Practical Allergy (PRACTALL) consensus report suggested the following definition: “Repeated attacks of airway obstruction and intermittent symptoms of increased airway responsiveness to triggering factors, such as

exercise, allergen exposure and viral infections” (9). A Nordic consensus report suggested a feasible way of diagnosing asthma in children: “Asthma can strongly be considered in the following circumstances: 1. From the third attack of airway obstruction during the last years is current asthma; 2. One attack of asthmatic symptoms occurring after the age of 2 years; 3. Irrespective of age in an attack in children with eczema, food allergy or other allergy; 4. If the child does not become free of symptoms when infection has ceased, or has persistent symptoms for more than one month.”(10). The timing of and criteria for diagnosing asthma diagnosis is debated, while for studies asthma-like diseases are often classified into viral wheeze and multiple-trigger wheeze, and further classified by transient, persistent and late-onset, starting after three years of age (11). In the Bronchiolitis study, we used the more objective term of recurrent bronchial obstruction (rBO), similar to the commonly used term “recurrent wheeze” in English speaking countries, as a surrogate marker of asthma at two years of age. Typical symptoms of asthma are wheeze, cough or breathlessness, varying over seasons and with different triggers, a personal or family history of atopic disorders (12). The medical history may or may not be accompanied by signs of bronchial obstruction reduced lung function or increased bronchial hyperresponsiveness (13).

Risk factors for asthma can be genetic, prenatal or arise in childhood.

Parental allergic asthma has been found to be a risk factor for asthma (14). Although more than 100 genes have been linked to asthma (15), there is no specific gene that has been implicated in all subjects with asthma.

Other risk factors are atopic dermatitis (14), male gender (for asthma in childhood) (16, 17), tobacco smoke exposure (17) and parental atopic rhinoconjunctivitis (18). In a study of 138 children who had been hospitalised for bronchiolitis before 6 months of age, published in 2017, S. Tormanen et al. found that maternal asthma was the only independent risk factor



recorded at inclusion or at 18 months of age for asthma when the children were 11-13 years of age (19), while atopic dermatitis earlier than 12 months of age and allergy in mothers were no longer significant after adjusting for maternal asthma. The German Multicenter Asthma Study (MAS cohort) reported that parents with allergies increased the risk of developing atopic multimorbidity (20) in the off-spring.

Prenatal risk factors include prenatal maternal stress (21), prenatal maternal tobacco smoke, mother's nutrition, with a lack of foods with anti-inflammatory properties, e.g. fish, fish oils and omega-3-fatty acids, vitamin E and zinc, antibiotics and emergency caesarean section (15).

Risk factors in childhood include lack of exclusive breastfeeding for at least three months, reduced lung function in the first weeks of life, low socio-economic status (for the morbidity of asthma), caregiver stress, some lower airway viral infections in infancy (in some children), with an uncertain role per se in the development of asthma (15). Acute bronchiolitis and pneumonia in infancy have been found to be risk factors for asthma, and hospitalisation for severe RSV bronchiolitis in infancy increases the risk of asthma and allergy at 18 years of age (22-24). One study has shown that in children with atopic dermatitis, behaviour problems at the age of 35 months increase the risk for asthma at 53 months of age (25). Others have found that childhood risk factors for asthma include low parental socio-economic status, antibiotic use, allergic sensitisation, male gender until 13-14 years of age, and female gender for asthma after 13-14 years of age (15), atopic dermatitis (14), male gender (for asthma in childhood) (16, 17) and tobacco smoke exposure (15, 17).

#### Atopic dermatitis

Atopic dermatitis is a common chronic skin barrier disease, affecting 15-25 % of children, also in Norway (26). Atopic dermatitis starts in 80% before 6 years of age (27). Atopic dermatitis is characterised by pruritus and recurrent eczematous lesions that are accompanied

by T-helper cell, Th2-dominated inflammation (28). The aetiology is multifactorial, with complex interactions between genetic and environmental factors, such as skin barrier dysfunctions, allergy/immunity and pruritus (28).

Risk factors for atopic dermatitis include parental allergic diseases, living in an urban setting, in regions with low ultraviolet light exposure, dry climate, a diet high in sugar and polyunsaturated fatty acids, repeated antibiotics exposure before five years of age, a small family size and high household educational level (27).

Atopic dermatitis in early childhood is associated with an increased risk of later asthma, allergic sensitisation, atopic rhinitis and food allergy, i.e. the child may be subject to “the atopic march”.

#### Allergic sensitisation

Allergic sensitisation can be detected by allergen-specific IgE or skin prick tests. Allergic sensitisation can occur without symptoms, and atopic disease is not necessarily associated with allergic sensitisation. Markers for an increased association with asthma or asthma development are being highly sensitised, early sensitised, especially to outdoor allergens, sensitisation to multiple allergens or to cats, dogs or horses, and late sensitisation to indoor allergens (29). The prevalence of early allergic sensitisation varies among countries, with recent rates based upon skin prick test around 12-15% around one year of life (30).

Development of allergy is possibly determined for many individuals by events occurring during pregnancy and the first years of life (31). Atopic dermatitis is found to increase the risk of later allergic sensitisation, whereas wheeze, asthma or rhinitis is not (32).

#### Allergic rhinitis

Allergic rhinitis is uncommon in young children. In the BAMSE cohort study of 2024 children, the proportion of children with allergic rhinitis rose from 5 % at age four to 14 % at

eight years of age. The comorbidity with other atopic diseases was high. Twenty-eight % of the four-year-old children with allergic rhinitis had asthma (33). Allergic rhinitis is not a topic of further discussion of this study, except for possible influences of parental allergic rhinitis.

### The concept of acute bronchiolitis

In these studies, we used the definition of acute bronchiolitis as described by Court (18, 34): *“Illness mainly affecting infants, especially in the first 6 months of life. Rapid respiration, dyspnoea, wheezing, chest recession, cough, rhonchi and rales are very frequent. Visible distension of the chest and increased pulmonary translucency on the chest radiograph are frequent and of high diagnostic significance. Upper respiratory features, especially nasal discharge and a red pharynx are frequent. Fever is very frequent, but high fever uncommon.”*

In 2006, the American Academy of Pediatrics and European Respiratory Society stated that bronchiolitis is a clinical diagnosis: “a constellation of clinical symptoms and signs including a viral upper respiratory syndrome followed by increased respiratory effort and wheezing in children less than 2 years of age” (35). Many recent studies from Europe and the USA have restricted acute bronchiolitis to children in their first year of life, as children hospitalised for wheezing between 12 and 24 months have a greater risk for asthma, with a different pathophysiology (36).

Acute bronchiolitis is the most common cause of hospital admission of infants worldwide (37, 38) and in Norway (39). The disease is characterised by acute inflammation, oedema and necrosis of epithelial cells covering the inner aspect of the small airways and increased mucus production. The disease usually starts with rhinitis and cough, proceeding to tachypnoea, wheezing, rales, use of accessory respiratory muscles, and/or nasal flaring (40). The most common causing virus is respiratory syncytial virus (RSV) (40).

Severe RSV bronchiolitis in children in their first year of life is associated with increased prevalence of asthma, clinical allergy and sensitisation to perennial allergens in 18 year-old subjects (23). Infant bronchiolitis has been associated with later reduced QoL in children (41). Increased rate of asthma, reduced QoL and irreversible airway obstruction has been shown in adults 30 years after hospitalisation for acute bronchiolitis before 24 months of age (22, 42). Whether acute bronchiolitis is a cause of asthma or there is a common relationship for acute bronchiolitis and asthma is unclear, but a randomised study of an antibody against RSV, palivizumab, given to prematurely born infants to prevent acute bronchiolitis by RSV, showed a reduced rate of days of wheezing in the first year of life, but did not comprise two year of age data.(43).

In a retrospective cohort study of 95310 children, an age of four months at the peak of the first winter viral season was associated with both clinical bronchiolitis and childhood asthma compared with 12 months earlier. (44). The severity of acute bronchiolitis was associated with young age, premature birth, secondary smoke, maternal smoking during pregnancy and low socio-economic status in a Brazilian study (45). In a Swedish study, the rate of hospitalisation was higher in the city areas with the highest social burden, but the severity of the disease was similar among the hospitalised children (46).

In the Bronchiolitis ALL South East Norway study providing the basis for this thesis, Skjerven et al. found respiratory syncytial virus (RSV) in 83% of the infants recruited with acute bronchiolitis in infancy. a high genomic load of RSV was associated with disease severity, whereas virus type or coinfection were not (47).

### General asthma risk factors

These may be sensitisation to aeroallergens, especially indoor allergens, secondary smoke and epigenetic mechanisms, nutrition, exposure to microbial products, male gender for asthma

until adolescence and female gender for asthma from adolescence (15) and obesity, especially in women (15).

Genes appear to play only a limited role in asthma development, thus genetic and epigenetic effects seem to interact with environmental factors including allergens, cigarette smoke, air pollutants and infectious agents during pre- and postnatal periods (48).

### Health-related quality of life (QoL) in young children

The patients' point of view and their satisfaction are important outcomes in preventive measures, interventions and disease treatment, often referred to as patient related outcomes (PRO)s. One such measure is quality of life (QoL). However, QoL may also be a marker of un-health and possibly a risk factor for later disease.

Global QoL includes aspects independent of people's health. In order to focus on the individuals' health, concepts of health-related quality of life have been developed. A wide variety of definitions of health-related quality of life (QoL) has been suggested. A basis for development of these concepts has been the World Health Organisation's definition of health as "a state of physical, mental and social well-being", not merely the absence of disease. A special challenge arose in the development of a health-related QoL concept in children. In young children, questions have to be answered partly by proxy, usually a caregiver, and in the youngest children, only by proxy. Hans M. Koot and Jan L. Wallander define QoL applicable for children and adolescents with chronic conditions this way: "Quality of life is the combination of objectively and subjectively indicated well-being in multiple domains of life considered salient in one's culture and time, while adhering to universal standards of human rights" (49). Tools for QoL measurement may be generic or disease specific. The last category is divided into functional status instruments and multidimensional instruments (49). Generic measures are appropriate for comparison between subjects with different diseases and between subjects with and without disease, but less specific for treatment comparisons. In

order to be appropriate for the children's stage of mental development, age-specific measures are developed. On the other hand, defining these measures for too short age periods will reduce the possibility to follow up QoL measurements in longitudinal studies. The generic QoL questionnaire The Infant Toddler Quality of Life Questionnaire™, ITQOL-97, is a clinical multidimensional outcome measure, with concepts (domains) of physical health, psychosocial health, health change, and impact on the parents and family. ITQOL-97 has been used in studies of wheeze and lower respiratory tract disease and to compare subjects with burn injuries, neurofibromatosis type I, functional abdominal complaints, undergone respiratory syncytial virus infection, wheezing illness and healthy subjects (41, 50, 51). The questionnaire is validated for children from two months to five years of age, and a validated translation into Norwegian was provided by the copyright owner before we were ready to take it into use. The behaviour domains, i.e. General behaviour, Overall behaviour and Getting along and of course the domain Change in health (compared to one year ago) are only applicable for children at least 12 months of age.

Factors that may influence QoL in young children may be chronic disease and psychosocial environment, individuals' capability to tackle disease and physical and mental stress, the individuals' temperament and interactions between the individuals and their environment.

#### Morning salivary cortisol

Cortisol is a steroid hormone synthesised in the zona fasciculata of the adrenal cortex, has a variety of effects, is important for preparing the body for action in times of danger and is an indicator of stress in adults, children and infants (52). Cortisol raises blood glucose in the initial phases of fasting by enhancing mobilisation of glycogen reserves, and in late phases of fasting by promoting lipolysis and inhibiting lipogenesis from glucose. Cortisol causes a shift toward Th2-mediated humoral immunity rather than general immunosuppression, and may

prevent tissue damage by Th1/pro-inflammatory cytokines and other products of activated macrophages in acute inflammation (53).

Corticotrophin releasing hormone (CRH) is secreted from the hypothalamus, carried to the pituitary gland by the hypothalamus-pituitary portal system, and stimulates secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary. ACTH stimulates the adrenal cortex to secrete cortisol and other glucocorticoids. This system is called the hypothalamus-pituitary-adrenal (HPA) axis. In addition, neuroendocrine pathways also stimulate the secretion of glucocorticoids. There is a negative feedback from cortisol and ACTH on the secretion of CRH, and from cortisol on the ACTH secretion (54). Acute stress leads to an increased secretion of cortisol by the HPA-axis. Animal and human studies have shown that the response of the HPA-axis to acute stress is blunted in subjects with allergy exposed to chronic stress (54). A reverse association between adrenal function and bronchial responsiveness has been shown in children with asthma, depending on the severity of the bronchial hyperreactivity (55). A study of 7-10 year old children showed that children exposed to recurrent maternal stress had an elevated cortisol level in response to an acute stressor if they did not have asthma, and reduced cortisol levels if they had asthma (3).

Cortisol can be measured in plasma, serum, urine, hair and saliva. 24-hour urinary cortisol sampling has the advantage of representing the HPA axis over a longer time interval, but will not express any peak value. Urinary cortisol is not only dependent on adrenal cortisol production, but also liver clearance and renal function. Urinary cortisol should preferably be measured together with urinary cortisol metabolites (52). A disadvantage of 24-hour urinary cortisol sampling is poor compliance (52).

To catch a cortisol awakening response (56), salivary samples should be taken within 60 minutes of awakening. This is more feasible to obtain by salivary cortisol sampling than by

blood sampling. The cortisol awakening response starts immediately after awakening and reaches a maximum 30 minutes after awakening in children and adolescents (57). The chance of obtaining a sample at the peak time of the cortisol circadian rhythm is likely to be greater when the sample is taken within short time after awakening than if one has to wait until a laboratory is open later in the morning. Waiting to a pre-set time after awakening may also be more difficult to perform within due time than if the sample should be taken as soon as possible after awakening, before the first meal. Salivary cortisol has been shown to decrease in 1-4 year-old children from 8:00 a.m. to 10:00 a.m. (58). The cortisol circadian rhythm is established in infancy and its start is associated with the start of a sleep-wake-rhythm (59, 60). Salivary cortisol mainly represents the free fraction of cortisol, and the free fraction is considered to exert the main part of biological activity in the target tissues (52).

One study showed higher morning salivary cortisol in children with recurrent abdominal pain, and in these children, morning salivary cortisol was highly correlated with serum cortisol (61).

The relationship between atopic disease development, QoL, stress and cortisol  
Psychological stress or stressful events can contribute to development or worsening of atopic disease (62, 63), and exposition to maternal stress beyond the postnatal period has been shown to predict an increase in plasma cortisol in seven to ten year-old children without asthma and a decrease in those with asthma (3). Reduced QoL has been reported in school children with asthma (64). Severity of atopic dermatitis in children is associated with QoL (65). The severity of asthma (66, 67) and atopic rhinoconjunctivitis (68) negatively influences QoL. Reduced QoL has been reported in adolescents with asthma exposed to second-hand smoke (69) and adults who were exposed to maternal smoking during pregnancy (70).

A broad concept, health related QoL, may in part be correlated with psychological and physical stress. Hyperactivity of the HPA-axis may be characteristic of early phases of allergy



development. This hyperactivity may influence the immunologic system in several ways, for example through the T helper (Th) cell responses, specifically Th1 and Th2 responses, which represent different immunologic processes (71, 72). Stress in asthma is thought to induce an attenuated responsiveness of the HPA-axis with a lower secretion of cortisol and thereby an increased production of cytokines typically counter-regulated by cortisol (62). The chronic stress and chronic inflammation frequently observed in atopic disease and the chronic inflammation may be associated with the reduced response of the HPA-axis as different pro-inflammatory cytokines inhibit the ACTH-induced production of cortisol (73) and an inadequate inflammatory response (71, 72). Reduced basal morning cortisol levels are found in children with asthma, also in children who do not use inhaled corticosteroids (54, 74). The reduced cortisol response to acute stress seen in chronic stress in children with asthma or atopic dermatitis (75), though with conflicting result in young children with reported for atopic dermatitis (76), is not seen in chronic stress in non-atopic children, being associated with a higher cortisol response (3, 77). The capacity for a high morning salivary cortisol can correlate with the capacity for mobilising cortisol in acute stress. The cortisol awakening response, likely to be represented in many of our morning salivary cortisol samples, is positively associated with job stress and general life stress and negatively associated with fatigue, burnout or exhaustion (78). A low function of the HPA-axis and the following low morning salivary cortisol may be associated with a low QoL due to asthma and chronic stress (74). On the other hand, a low stress level in children with asthma and possibly other atopic disease will probably be associated with higher morning cortisol than a high stress level in children with asthma. A low stress level in children with asthma is probably also associated with higher QoL. Accordingly, in children with asthma and possibly other atopic disease, morning cortisol may be lower in children with low QoL and higher in children with high

QoL. Salivary cortisol sampling is feasible, does not induce acute stress by itself, as opposed to blood sampling (75) and can be taken in the homes at selected time points.

Knowledge gaps in the relationship between asthma and asthma risk factors including bronchiolitis, and chronic stress, represented by cortisol and QoL.

It has not been known if acute bronchiolitis in infants younger than one year of age is associated with reduced QoL. Likewise, it is unclear to which extent severity of acute bronchiolitis in infancy, as measured by a clinical score at inclusion (79, 80), oxygen saturation, length of hospital stay or the need for supportive treatment (nasogastric tube feeding, extra oxygen supply or ventilatory support) affects QoL in infants.

It has not been known if stress as measured by morning salivary cortisol is associated with acute bronchiolitis or development of asthma or other atopic diseases.

There are knowledge gaps about the degree of connection between asthma risk factors, atopic diseases and reduced QoL in children up to 24 months.

It has not been known whether hospitalisation for acute bronchiolitis influences the tendency from common asthma risk factors to reduce QoL.

To the best of my knowledge, it is not known if cortisol as a marker of stress is associated with acute bronchiolitis, and if acute bronchiolitis in infancy plays a role for an association between QoL and asthma or other atopic disease development in early childhood.

It has not been known if cortisol is associated with QoL, as a proxy for chronic stress, in early childhood, if a possible association is influenced by recurrent bronchial obstruction, as a proxy for asthma, or if a possible association is influenced by acute bronchiolitis in infancy.

## HYPOTHESES, OBJECTIVES AND SPECIFIC AIMS

### Hypotheses

We put forward the following hypotheses:

1. Acute bronchiolitis and severity of the disease are associated with reduced QoL.
2. Stress as measured by morning salivary cortisol is associated with acute bronchiolitis and with development of asthma or other atopic diseases.
3. QoL is associated with asthma or asthma development, risk factors for asthma and other atopic diseases in young children, with or without acute bronchiolitis in infancy.
4. There is a link between QoL, morning salivary cortisol and atopic diseases that is influenced by moderate to severe acute bronchiolitis in infancy.

### Objectives

Our main objective was to explore how QoL and salivary cortisol as a surrogate marker for stress may contribute to the development of asthma and atopic diseases in young children with or without acute bronchiolitis in infancy.

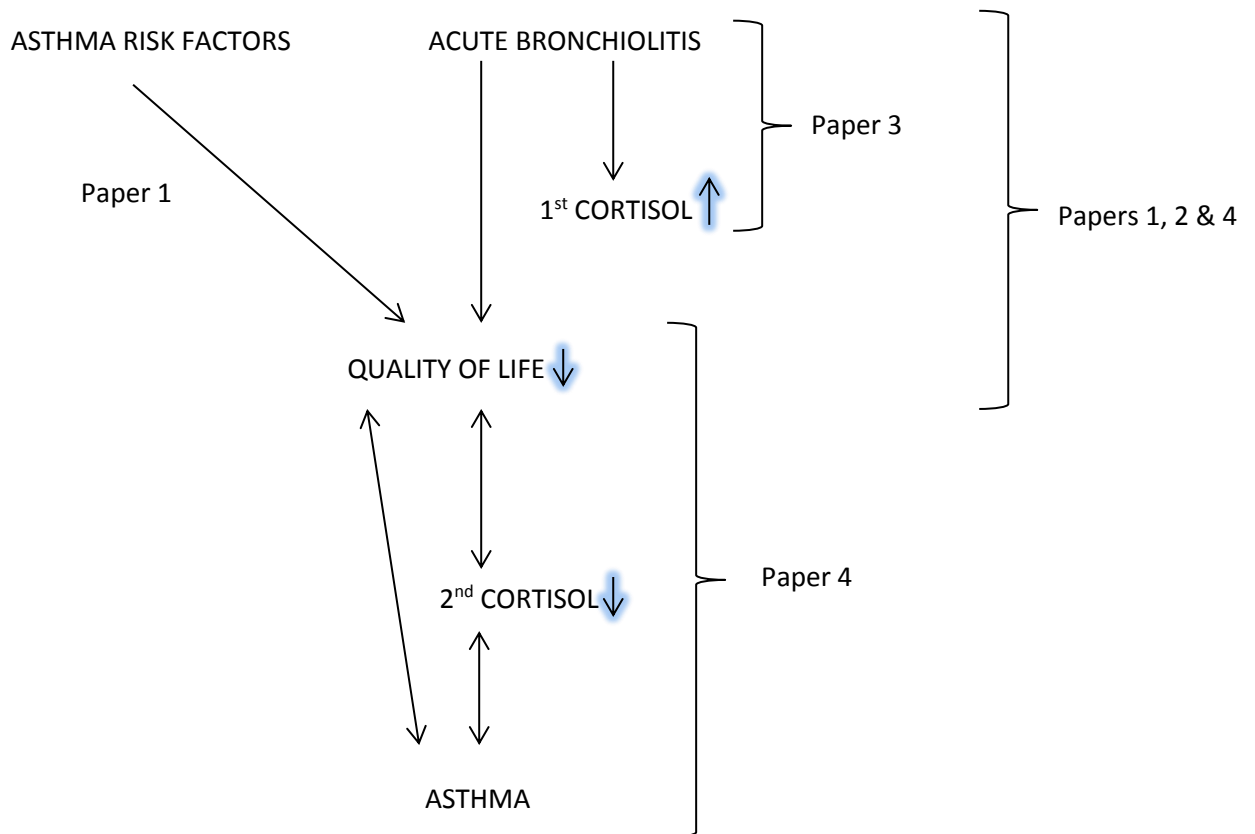
### Specific aims

Our specific aims were as follows:

1. To investigate if the acute bronchiolitis in infancy and severity of the disease are associated with reduced QoL in young infants (papers 1, 2 and 4).
2. Using salivary cortisol as a marker of stress; to determine if stress is associated with acute bronchiolitis and/or development of asthma or other atopic diseases (papers 3 and 4).
3. To determine if QoL is associated with asthma risk factors, asthma and/or other atopic diseases in young children with or without acute bronchiolitis in infancy (papers 1,4).

- To determine if there is a link between QoL, salivary morning cortisol and atopic diseases, and if such a link may be modified by acute moderate to severe bronchiolitis in infancy (paper 4).

**Figure 1. Schematic view of the topics of the papers. Blue marked arrows indicate increase or decrease.**



## METHODS AND SUBJECTS

### Study design

A cohort of 644 children from the South-East Health Region of Norway constituted the data source for the studies. One group of children, hereafter named the bronchiolitis group (n=404), were recruited for a randomised treatment trial of acute bronchiolitis in the first year of life, performed as a multicentre trial in eight paediatric departments of hospitals in the health region (79). They were recruited at hospital admission for moderate to severe acute bronchiolitis from January 2010 through May 2011. A group for comparison, hereafter named the control group, was recruited from the general population, by an invitation letter sent to the caregivers (in the following also termed the parents) of 3000 randomly selected children younger than 12 months of age from the municipalities Oslo and Fredrikstad. The parents of 240 children accepted the invitation, and they were enrolled and examined at Oslo University hospital, Ullevål, and Østfold Hospital Trust, from March through July 2012.

Inclusion criteria for the bronchiolitis group were moderate to severe acute bronchiolitis at admission to hospital and age below 12 months and sufficient Scandinavian language skills to comply with the study, while exclusion criteria were any glucocorticoid treatment in the preceding four weeks, more than one previous episode of bronchial obstruction, more than four weeks of lower airways symptoms or any severe or chronic disease that might significantly influence the progression of acute bronchiolitis. Inclusion criteria for the controls were age 0-12 months at the time of invitation. Exclusion criteria were any severe underlying disease, e.g. heart, lung, immunological, neurological or oncological disease.

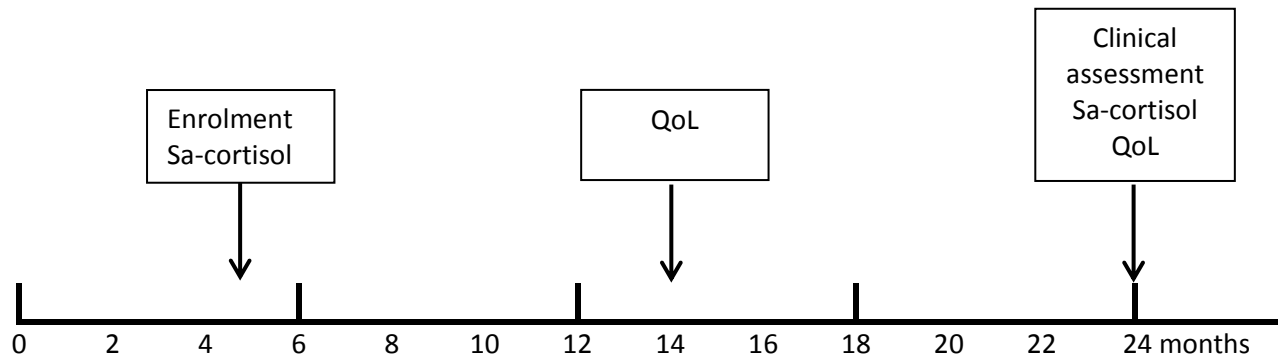
At inclusion, a structured interview including the child's disease history, family history and socio-economic data was performed focusing on symptoms on airway and atopic disease, followed by clinical examinations, sampling of biological material, including blood samples

and saliva for cortisol measures. The morning salivary cortisol samples were taken in the hospital for the bronchiolitis group and in the homes for the control group.

Nine months after inclusion, QoL (ITQOL) forms were sent to the parents of the 644 initially participating children. ITQOL was answered from December 2010 through January 2012 for the bronchiolitis group and from November 2012 through April 2013 for the control group.

At two years of age, the infants were invited to a follow-up examination, including parental interview, skin assessment and further clinical examination, skin prick testing for allergy and blood sampling. Additional sampling for salivary cortisol as well as ITQOL questionnaires were sent by mail in advance and brought to the clinics at the visits using the same standard procedures as for the inclusion visit. If forgotten, the parents received new questionnaires with return envelopes at the visit.

The two-year follow-up assessments and QoL ratings took place from September 2011 through December 2012 for the bronchiolitis group and from September 2013 through January 2014 for the control group. Specially trained physicians and nurses were involved. Again, a structured interview including family and personal disease history and socio-economic data was performed, focusing especially on airway and atopic disease. The children were checked for Hanifin and Rajka criteria for atopic dermatitis (81), and if a rash was found, severity of atopic dermatitis was assessed by a physician and a nurse by The Scoring Atopic Dermatitis (SCORAD) index (82). The children were skin prick tested for aeroallergens and food allergens. See Figure 2 indicating the children's mean ages at the examinations and surveys, except that the children of the control group's mean age was 6 months at enrolment. The mean age at the first QoL survey was 13.7 months in the bronchiolitis group and 14.4 in the control group. Mean ages at the second clinical examination and QoL survey was 24 months for both groups.

**Figure 2. Mean ages at the assessment time points.**

### Ethical considerations

This is an observational sub-study of the already approved Bronchiolitis All SE-Norway study, but with additional observations, i.e. QoL measurements by questionnaires to the parents. The scientific value of this sub-study was strengthened by our use of validated QoL questionnaires. Before enrolment, all caregivers signed informed written consents. The study was approved by the Norwegian Data Protection Authority and the Regional Committee for Medical and Health Research Ethics and registered in the Norwegian bio bank registry. The randomised clinical trial part of the study was registered in ClinicalTrials.gov, no. NCT00817466 (79). The additional observations were approved by the Regional Committee for Medical and Health Research Ethics, by a letter, document no. 2010/2563b, and new informed consents from the caregivers were given.

### Subjects

Eight of the 240 children recruited for the control group were analysed among the children enrolled in the bronchiolitis trial in the study present in paper 1, as they had been hospitalised for acute bronchiolitis before inclusion. In paper 2, only children from the original bronchiolitis group were included, as they were the only ones with data of acute disease

severity. For presentation of reference values of morning salivary cortisol in paper 3, only children recruited at hospitalisation for acute bronchiolitis, and accordingly with first morning salivary cortisol taken at hospitalisation, were analysed as children with previous bronchiolitis, and children from the original control group were analysed as reference children. The results of paper 3 also included subjects without QoL data. For simplification of the study groups and flow chart, for the study presented in paper 4, only subjects from the original bronchiolitis group were analysed as previous bronchiolitis subjects, and the three children with both QoL<sub>2</sub> and cortisol data previously hospitalised for acute bronchiolitis among the controls were analysed as controls. A simplified flow chart shown in Figure 3, from paper 4, shows how many of the subjects with both QoL (QoL<sub>2</sub>) and cortisol data at two years of age who had QoL<sub>1</sub> data, at an average of 14 months of age, nine months after inclusion, i.e. at the first survey, and how many subjects who had both sets of data at two years of age.



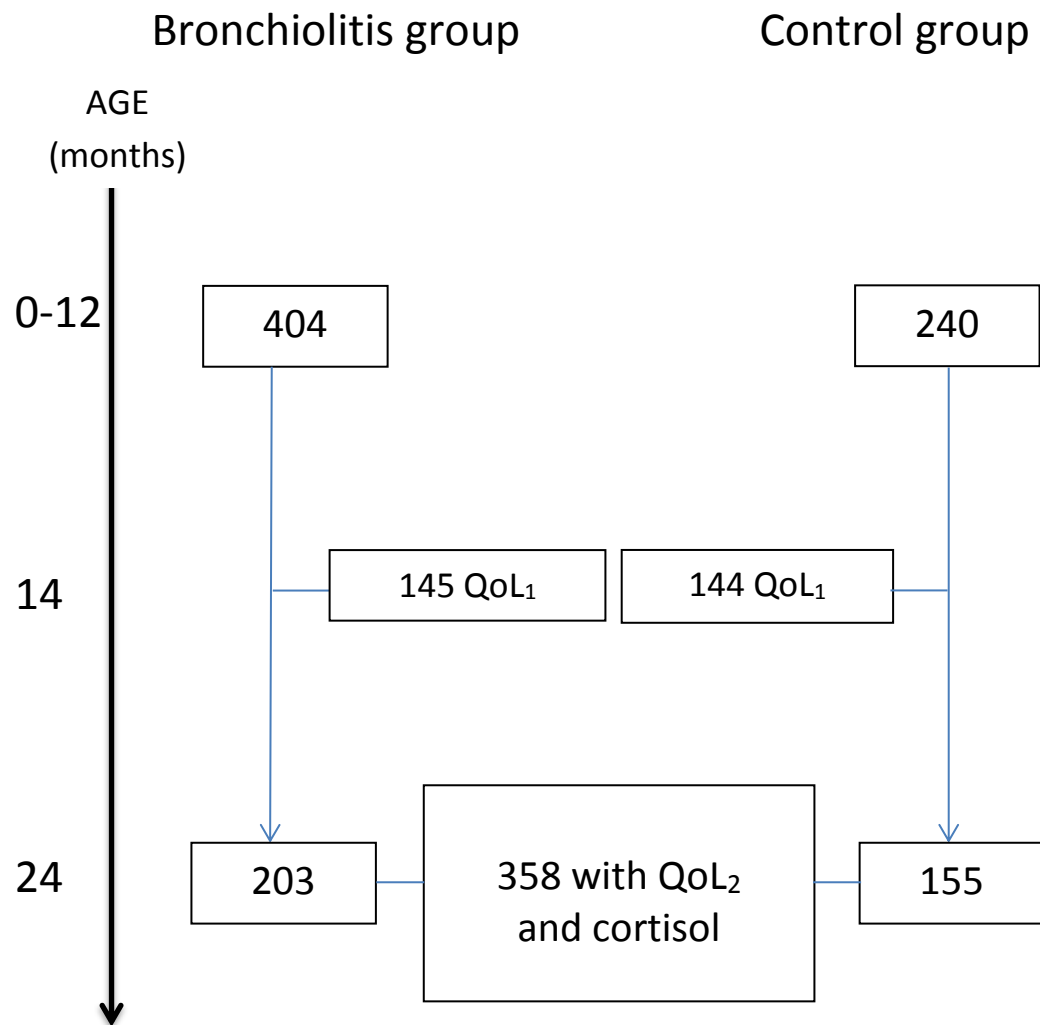
**Figure 3**

Figure 3 outlines the number of infants enrolled in the Bronchiolitis All SE-Norway study (top, n=644) who were subsequently included in the study presented in paper 4 (n=358) for analyses based upon available Quality of life (QoL) and/or salivary morning cortisol at 24 months of age. The QoL questionnaires were completed nine months after enrolment at approximately 14 months of age (QoL<sub>1</sub>) as well as at the time of the clinical examination at 24 months of age (QoL<sub>2</sub>).

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Additionally, paper 1 included also subjects without both QoL and cortisol results at two years of age, while eight children with reported hospitalisation for acute bronchiolitis before inclusion in the control group were analysed as children from the bronchiolitis group. Thus, the total of subjects with previous bronchiolitis and QoL results from the first survey was 217, and the number of control subjects was 198.

Demographic data and characteristics of the children are shown in Table 1. The bronchiolitis group includes the eight children from the general population with QoL data nine months after inclusion who had been hospitalised for acute bronchiolitis before inclusion, and the remaining children constitute the control group. At two years of age, in analysis of children with both QoL and cortisol, the bronchiolitis group included only those who were included when they were hospitalised, whereas three children in the control group had been hospitalised for acute bronchiolitis before inclusion. The groups differed mostly by the same variables at both time points. At two years, the mean age was 24 months for both groups. The average length of the children in the bronchiolitis group was 1.7 cm shorter. Second-hand smoke in infancy had been higher in the bronchiolitis group, 14.5% vs. 5.3 %. However, at two years, second-hand smoke exposure was only 2.5 % in the bronchiolitis group and 0.7% (in one child's home) in the control group. The number of subjects with rBO was 48.3 % in the bronchiolitis group and 14.2 % in the control group ( $p < 0.001$ ). The groups did not differ by frequencies of atopic dermatitis or allergic sensitisation, except for a higher frequency of mild atopic dermatitis in the bronchiolitis group, 44.2 % vs. 26.3 %. Among all subjects with rBO, the rate of inhaled corticosteroids did not differ significantly between the groups, 33.7 % vs. 22.7 % for those with salivary cortisol and QoL data at two years of age.

In the study for cortisol reference values, parental allergic rhinitis was higher among children of the reference (control) group, 37.7 % vs. 29.3 %.

**Table 1:** Characteristics and asthma risk factors at inclusion, of the subjects who had QoL<sub>1</sub> available. The Bronchiolitis group constituted 209 children included at hospitalisation as well as eight children from general population with QoL data hospitalised for bronchiolitis before inclusion, while the Control group constituted the 198 children recruited from the general population, not including the eight children with reported hospitalisation for acute bronchiolitis prior to enrolment.

	<b>Bronchiolitis group N=217</b>	<b>Control group N=198</b>	<b>All children N=415</b>
Boys n (%)	129 (59.4)	110 (55.6)	239 (58)
Age months inclusion, mean (SD)	4.1 (2.8)***	6.4 (3.4)	5.2 (3.3)
Birth weight, kg, mean (SD)	3.5 (0.6)	3.6 (0.6)	3.5 (0.6)
Birth length cm, mean (SD)	49.9 (2.9)**	50.6 (2.3)	50.2 (2.7)
Weight inclusion, mean (SD)	6.5 (1.9)***	7.8 (1.7)	7.1 (1.9)
Length inclusion, mean (SD)	62.2 (6.7)***	67.6 (6.2)	65.3 (6.9)
Education mother <sup>1</sup> , mean (SD)	4.1 (0.9)***	4.6 (0.7)	4.3 (0.9)
Education father <sup>1</sup> , mean (SD)	3.9 (1.0)***	4.3 (0.9)	4.1 (0.9)
Income mothers <sup>2</sup> , mean (SD)	2.0 (0.7)**	2.2 (0.7)	2.0 (0.7)
Income fathers <sup>2</sup> , mean (SD)	2.4 (0.6)**	2.6 (0.6)	2.5 (0.6)
Caucasian mother, n (%)	204 (94.9)	190 (96.0)	394 (95.4)
Caucasian father, n (%)	206 (96.7)	186 (93.9)	392 (95.4)
Breastfeeding in infancy n (%)	136 (73.9)	133 (75.1)	269 (74.5)
Second-hand smoke infancy n (%)	32 (16.7)***	(3.1)	38 (9.9)
Atopic dermatitis before incl. n (%)	23 (11.1)	21 (10.6)	44(10.9)
Parental asthma, n (%)	62 (29.5)	60 (30.3)	122 (29.9)
Parental AD, n (%)	61 (28.4)**	82 (41.4)	143 (34.6)
Parental atopic rhinoconjunctivitis, n (%)	121 (56.0)	118 (59.6)	239 (57.7)

<sup>1</sup>No school completed=1; primary school=2, secondary school=3, higher education ≤ 3 years=4, higher education > 3 years=5

<sup>2</sup>Annual income before tax, asked at the two-year-of-age follow-up: 1: <300.000 NOK. 2: 300.000-500.000 NOK. 3: >500.000 NOK.

Significance of differences between the groups: \* p<0.05\*\* p<0.01 \*\*\*<0.001

## Methods

### Interview

The parents underwent a structured interview guided by a physician of the including department of paediatrics, or a specially trained doctor for the general population children.

The interview included questions about the patient's and family's medical history with a special focus on atopic or obstructive airways disease, and questions about socio-economic factors. The interviews were repeated at the follow-up examinations by trained study physicians. For the general population subjects and for all children at the follow-up, the interviews also had a special focus on atopic dermatitis.

### Quality of life

See also General introduction and the appendix: ITQOL survey profile, provided by the trademark holder, HealthActCHQ Inc. We applied The Infant Toddler Quality of Life Questionnaire (ITQOL™), with copyright holder HealthActCHQ Inc., Boston, USA, consisting of 13 domains (concepts) and 97 questions in the version ITQOL-97. Crucial for our choice of ITQOL-97 was that when our project was about to start, this was the only generic QoL clinical outcome measure that had been validated in children younger than 12 months of age. There is no overall score. The domains General behaviour, Overall behaviour, Getting along and Change in health are only designed and were only used for children from 12 months of age. The domains have a lowest possible score, indicated the lowest QoL, of 0 and a maximum possible score of 100, meaning that one point score difference equals one percentage point difference. Originally, the Change in health score was rated from 1 through 5 (best). With permission from the copyright holder and in accordance with others (83), the Change in health score was recoded from 1 through 5 into 0-100. A score of 50 for Change in health indicates unchanged health compared with one year earlier.

The domain *Change in health* was exceptional in the original version, with scores ranging from one through five, and can be treated as a categorical variable. Robust regression by

Huber's M method does not allow the dependent variable to be categorical. To exclude a type 2 error, we tried to see if there were significant findings by analysing cortisol at two years of age as a dependent variable, with Change in health at 14 and 24 months of age respectively, as a categorical variable, selecting the best scores as reference due to few subjects with lowest scores – much worse than one year ago. We adjusted for age and gender.

#### Severity of acute bronchiolitis

The hospitalised children underwent clinical examinations routinely used for children to be admitted acutely, and the same examinations were performed for the general population children. Disease severity was reported by several measures.

The length of stay (LOS) from intention-to-treat analyses in the randomised clinical trial (79) was recorded based upon each individual hospital chart.

Supportive treatment, i.e. oxygen supplement, nasogastric tube feeding or ventilatory support, was recorded daily in CRFs. The severity by supportive treatment was categorised into three groups: 1. No supportive treatment. 2. Supportive treatment, but no ventilatory support. 3. Ventilatory support, regardless of other supportive treatment.

The severity of acute disease at admission was rated by a score form giving scores of 0-10 (maximum severity) as described by Kristjansson (79, 80).

Oxygen saturation (SpO<sub>2</sub>) was measured by pulse oximeters routinely used in the clinics.

#### Morning salivary cortisol

Parents took saliva samples on the first morning after hospital admission of the children included during hospitalisation, or at home and brought the samples to the clinic for the control children and before the follow-up visits. The parents were told to take the samples as soon as possible after the first awakening after 06:00 a.m., before the first meal. Two small, arrow-shaped hydrocellulose microsponges (Sorbette®, Salimetrics Europe Ltd., Suffolk,

CB8 7SY, UK), measuring 0.7 x 1.8 mm, attached to plastic applicator shafts, were introduced into the child's mouth, preferably under the tongue. They should be kept within the mouth for 60-90 seconds, until the microsponges were soaked with saliva. If necessary, the microsponges could be taken out and reinserted into the mouth (84). The salivary samples were put into their standard containers and brought to the investigation site, where they were frozen at -86°C until transferal to Karolinska Institutet, Stockholm, Sweden, for analysis. Radioimmunoassay was performed according to the manufacturer's instructions with kits from Cisbio Bioassays (Codolet, France) with monoclonal rabbit antibodies binding cortisol. In each test tube was a known amount of cortisol labelled with <sup>125</sup>I in Tris buffer, competing with the salivary cortisol to be attached to the antibody binding sites. The saliva was kept in a water bath at 37°C in the tubes for 30 minutes, and the poured out. Afterwards, the tubes were rinsed with a predefined amount of water and left upside down for a period before they were put into a gamma counter. A cortisol calibrating sample in the kit provided data for a standard curve for comparison. The method has a working range of 0-2000 nmol/L. 150 µL of saliva is required. Analytical sensitivity is 3.0 nmol/L.

#### Recurrent bronchial obstruction and asthma

Obstructive airways symptoms were recorded from the two-year-of-age follow-up parental interviews as the total number of parent-reported wheezing episodes. For those hospitalised for acute bronchiolitis, the acute illness was counted as one episode. Results from inclusion were checked to avoid that episodes reported at inclusion were forgotten at the follow-up examination interview. Asthma is challenging to diagnose in early childhood. The above mentioned Nordic consensus guideline (10) is the basis for our definition of a proxy for asthma, recurrent bronchial obstruction (rBO) as at least three episodes of bronchial obstruction at two years of age, as we also applied in a previous study (85). The total number of wheezing episodes was used as a severity measure in some analyses.

### Atopic dermatitis

At inclusion, diagnosed atopic dermatitis (in paper 1 called atopic eczema) as reported by parents was applied for analyses. At two years of age, atopic dermatitis was diagnosed by examination and interview by modified Hanifin and Rajka's criteria (81), with a few modifications for practical reasons, see paper 4, supporting information. Severity of atopic dermatitis was scored by the Scoring AD (SCORAD) index (82) by a trained physician as well as a trained nurse, and the higher of the two scores was selected. The reason for selecting the highest score could have been arbitrary, but we supposed it likely that a more thorough examination had been completed in the case of the higher score. In the cases where only a nurse or only a physician had scored the severity, these scores were used for analyses.

Children without signs of atopic dermatitis were not scored. Accordingly, having no SCORAD points was set to zero in the statistical analyses, to form a basis for comparisons.

### Allergic sensitisation

This was defined as at least one positive (mean wheal diameter at least 3 mm more than the negative control) of the following skin prick tests: dog dander, cat dander, house dust mite (*Dermatophagoides pteronyssinus*), birch, timothy and mugwort pollen, the mould species *Cladosporium herbarum* and *Alternaria tenuis*, hen's egg white, peanut, almond, hazelnut, wheat, cow's milk, soy, cod, shrimp and positive and negative controls (Soluprick, Soluprick SQ, ALK, Hørsholm, Denmark), performed according to EAACI guidelines (86, 87). The skin prick tests were performed according to the GA<sup>2</sup>LEN criteria (88). The borders of the wheals were marked by a pen and transferred to sheets of paper by transparent tape. The wheal size was calculated as the mean of the greatest diameter and the greatest diameter measured perpendicularly on the greatest diameter. The sum of allergen wheal diameters exceeding negative control were included in disease severity analyses (85).

### Asthma risk factors

We selected parental asthma, parental atopic rhinoconjunctivitis, second-hand smoke and atopic dermatitis in infancy as asthma risk factors to study their impact on QoL nine months after hospitalisation for bronchiolitis in infancy or inclusion of control subjects.

### Statistical analysis

For comparison of background characteristics, continuous normally distributed data were analysed by student's T-test, and non-normally distributed data were analysed by the Welch test unless otherwise indicated in the tables. Categorical data were assessed for group differences by Pearson's chi-square test. Due to non-normality of the distribution of the QoL scores and cortisol results and their residuals, analyses of QoL and cortisol data were made by Huber's M method of robust regression (89). The relative impact of each asthma risk factor on the ITQOL domain was tested using Hosmer's backward elimination technique in robust regression analysis, but keeping age and gender in all models (90). The level of significance was set to  $p=0.05$  for all analyses. For assessment of reference values for morning salivary cortisol (paper 4), percentiles were estimated.

When evaluating the clinical significance of differences of QoL scores, a concept of minimal clinically important difference or may be relevant. We have not calculated minimal clinical important differences for our own studies, but refer to a study, the Generation R study, of 5000 children about QoL and asthma-like symptoms at 12 months of age (50). In analysis adjusted for infant and maternal characteristics, the researchers found a percentage point difference in QoL scores between severe symptoms and no symptoms of 6.5 for Temperament and moods, 8.2 for Bodily pain/ discomfort, 9.4 for General health, 5.3 for Parental impact – emotions and 7.0 for Parental time, whereas, after adjustments for background variables, no significant differences were found between severe and no symptoms for Physical functioning, Growth and development and Family cohesion.



By univariate analysis, a percentage score difference in QoL scores between severe symptoms and no symptoms were 9.1 for Physical functioning, 5.8 for Growth and development, 9.3 for Temperament and moods, 14.9 for Bodily pain/ dysfunction, 16.8 for General health, 8.9 for Parental impact – emotions, 9.9 for Parental impact – time and 2.5 for Family cohesion. Effect sizes indicated a moderate difference for Growth and development, Temperament and moods, Parental impact – emotions and Parental impact – time between subjects with severe and no asthma-like symptoms. For General health, effect sizes indicated a moderate difference in scores between subjects with moderate and no symptoms, with a difference of 8.0 by univariate analysis and 3.9 in adjusted analysis. For Bodily pain/ discomfort, General health and Family activity, a great difference was found between subjects with severe and no symptoms of asthma-like disease.

We did not correct for multiple variables, as the ITQOL domains are not independent from each other. Interaction was analysed by multiple regression with two-way analysis, eliminating non-significant products of variables by Hosmer's backward elimination technique. Interaction was found between bronchiolitis and some of the asthma risk factors in analyses with some of the QoL domains as dependent outcomes. Likewise, interaction was found between cortisol and bronchiolitis with some of the QoL domains as outcomes. Hence, analyses of QoL comparing the groups were stratified for the bronchiolitis and control groups.

Confounding by socio-economic variables was tested by multiple regression and the Hosmer's backward elimination technique, but retaining age and gender in the analyses. Interaction analyses with maternal education, maternal ethnicity (Caucasian or not), prematurity, age and gender in the analyses of associations between bronchiolitis severity and QoL was performed first by selecting variables by multiple robust regression and Hosmer's stepdown procedure, retaining age and gender in the analyses, leaving variables with a p-value of less than 0.3 for two-way analysis by multiple robust regression and Hosmer's

stepdown procedure, then eliminating products with p-values more than 0.05. Confounding was considered significant if the association was changed by at least 25 % (90). The analyses of associations between QoL nine months after inclusion and asthma risk factors were performed with the Number Cruncher Statistical System (NCSS Kaysville, Utah, USA) version 2007 and the SPSS PASW Statistics 18 (IBM Corporation, Armonk, New York, USA).

The analyses of associations between QoL nine months after inclusion and severity of acute bronchiolitis in infancy were performed with the IBM SPSS Statistics 20 (IBM Corporation, New York, USA) and NCSS 2007 (see above).

For analysis of reference values of morning salivary cortisol and comparison of morning salivary cortisol between children hospitalised for acute bronchiolitis in infancy and controls, percentile analyses and robust regression were performed with NCSS 2007 (see above).

Otherwise, IBM® SPSS® (Armonk, New York, USA), version 22 was applied.

Analyses of associations between morning salivary cortisol and QoL, between atopic disease and morning salivary cortisol and between atopic disease and QoL were performed with IBM Statistics 21 (IBM Corporation, Armonk, New York, USA) and the Number Cruncher Statistical System (NCSS Kaysville, Utah, USA), version 11, and for analysis of background characteristics of this study, IBM SPSS Statistics 25.0 was applied.

## RESULTS

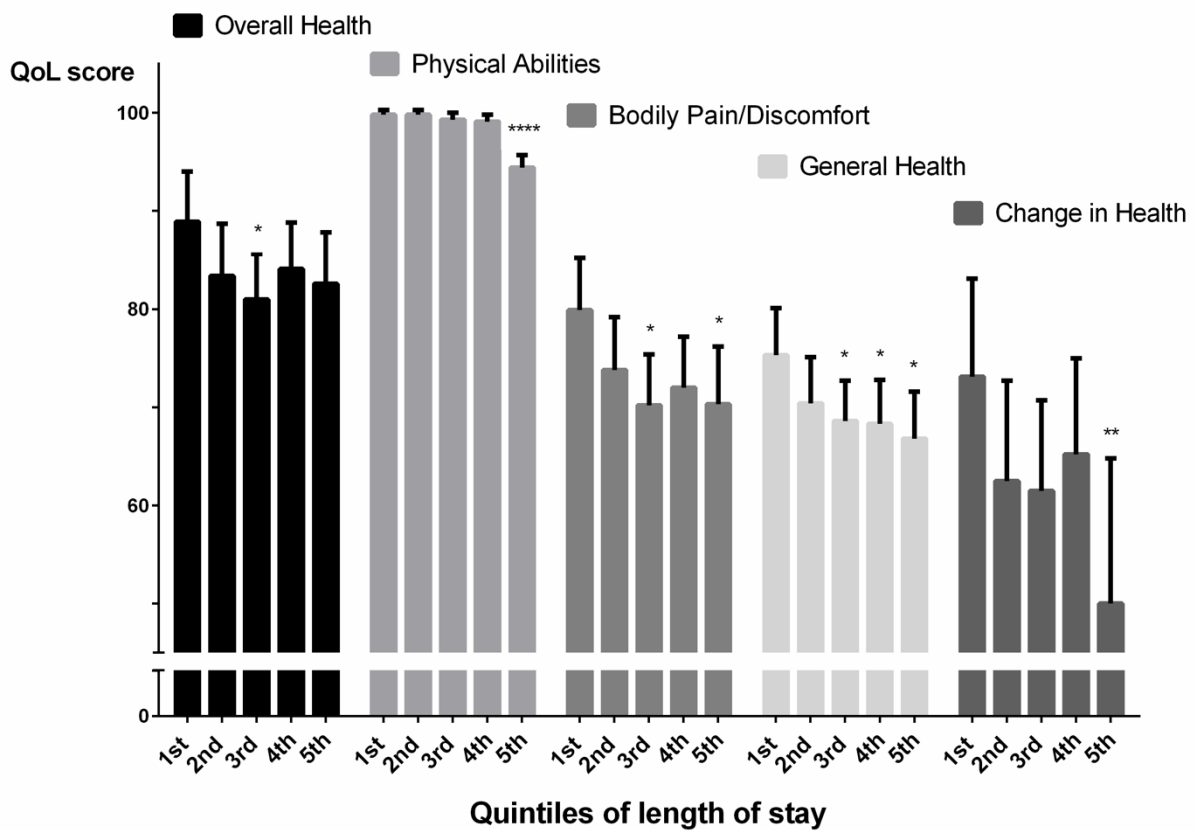
Are acute bronchiolitis in infancy and severity of the disease associated with reduced QoL in young children? (Papers 1, 2 and 4)

The QoL given as weighted mean scores nine months after inclusion, at a mean age of 14.0 (SD 3.3) months, was significantly reduced for the bronchiolitis group compared with the control group for two domains, with scores for Overall health of 85.4 (95% CI 83.6, 87.2) versus 91.2 (95% CI 89.4, 93.0), respectively and the corresponding scores for General health of 70.2 (95% CI 68.5, 71.9) versus 81.8 (95% CI 80.0, 83.5). The domain Change in health compared with one year ago was increased among the bronchiolitis group compared to controls (56.0 (95% CI 48.9, 53.8) versus 51.4 (95% CI 48.9, 53.8) nine months after inclusion. (All p-values < 0.0001). Significant differences were not found for the other domains.

At two years of age, mean age (range) 24.2 (17.6-34.7) months, on the average 10 months after the first survey, the differences between the groups were largely similar, with QoL significantly reduced for the bronchiolitis group compared with the control group for the domains Overall health, with a weighted mean score of 83.4 (95% CI 81.3, 85.5) versus 88.7 (95% CI 86.3, 91.1),  $p < 0.01$ , and General health, with a weighted mean score of 67.1 (95% CI 65.0, 69.2) versus 78.3 (95% CI 75.9, 80.7),  $p < 0.0001$ , and increased for the domain Change in health compared with one year ago, with a weighted mean score of 65.2 (95% CI 62.8, 67.8) versus 56.9 (95% CI 54.1, 59.7),  $p < 0.0001$ . Significant differences were not found for the other domains.

Nine months after the acute bronchiolitis, increased disease severity given as LOS and need for supportive treatment, was associated with lower QoL. Figures 4 and 5 show the unadjusted associations between QoL of LOS and need for supportive treatment, respectively.

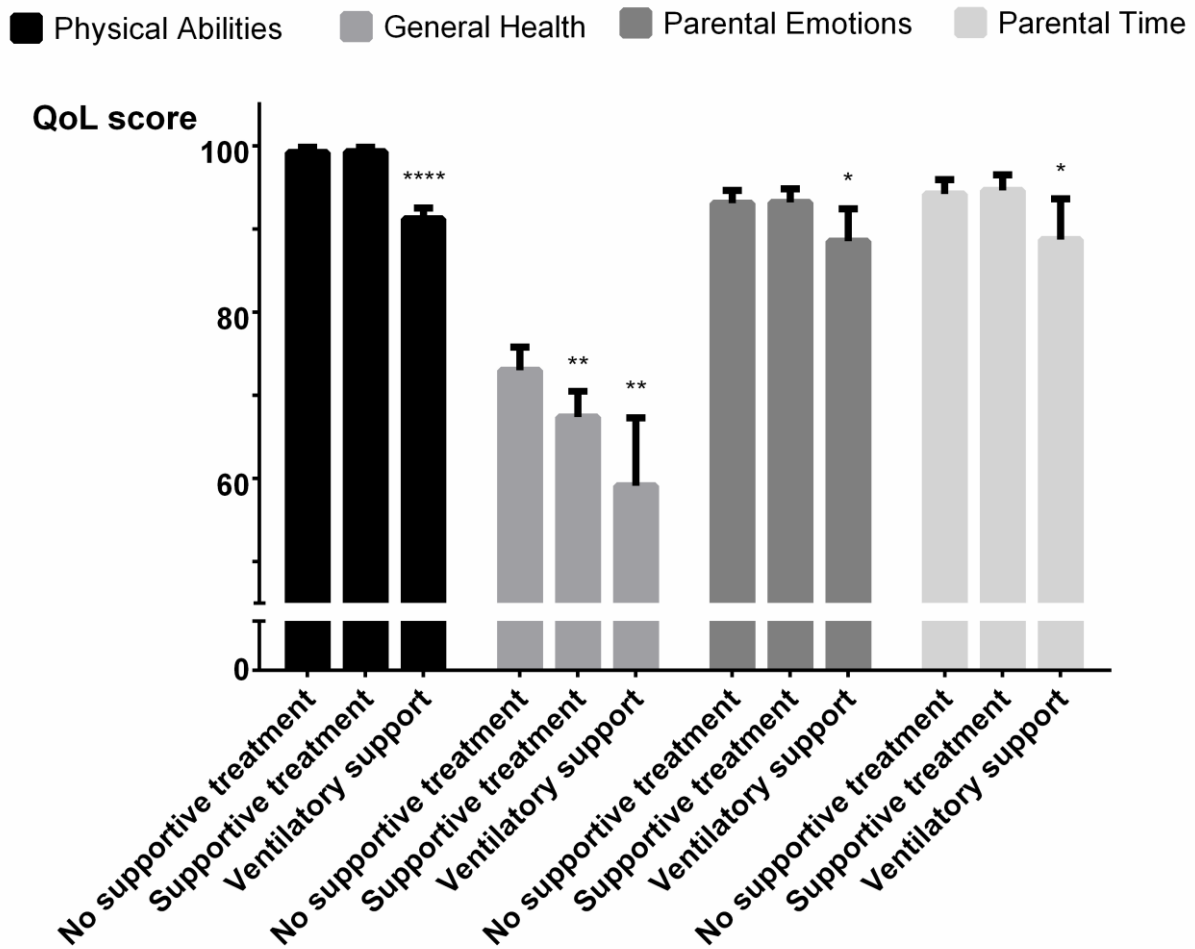
**Figure 4 Length of stay quintiles and QoL**



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Quality of life is given by domains with statistically significant associations with bronchiolitis severity based upon the quintiles of length of stay. Weighted mean scores are shown for each quintile.

\* p<0.05. \*\* 0.001<p<0.01 \*\*\*\* p<0.0001.

**Figure 5 Bronchiolitis severity by need for supportive treatment and QoL**

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Quality of life is given by domains with statistically significant associations with bronchiolitis severity based upon the need for supportive care. Supportive care: Supplementary oxygen or nasogastric tube feeding, but not ventilatory support. Weighted mean scores are shown for each quintile.

\*  $p < 0.05$ . \*\*  $0.001 < p < 0.01$  \*\*\*\*  $p < 0.0001$ .

Table 2 shows that LOS was negatively associated with QoL for five domains in analyses adjusted for age and gender, while one episode of BO before inclusion, did not alter the associations for most domains. The only exception was that the association between LOS and Bodily pain/ discomfort was no longer statistically significant after adjusting for one prior BO

episode. Table 3 shows that the need for ventilatory support was negatively associated with four domains of QoL nine months after inclusion, in analyses adjusted for age, gender and one previous episode of bronchopulmonary obstruction.

**Table 2:** The table shows significant associations between disease severity given as Length of Stay (LOS) and QoL domains, 1<sup>st</sup> quintile as reference, only domains with p<0.05, (95% CI) with adjustments for either age and gender, or for age, gender and one previous episode of bronchial obstruction (BO).

QoL domain	Adjustments	1 <sup>st</sup> quintile (0.5-23.0 hours) N=38	2 <sup>nd</sup> quintile (23.0-47.9 hours) N=39	3 <sup>rd</sup> quintile (47.9-75.6 hours) N=51	4 <sup>th</sup> quintile (75.6-124.9 hours) N=43	5 <sup>th</sup> quintile (124.9-408.1 hours) N=38
Overall health	Age, gender	88.9 (83.9, 94.0)	-4.6 (-11.3, 2.2)	-6.4 (-12.6, -0.1)*	-3.6 (-10.0, 2.8)	-5.6 (-12.5, 1.3)
	Age, gender, 1 BO	92.0 (85.7, 98.3)	-6.0 (-12.6, 0.5)	-4.1 (-10.2, 1.9)	-4.0 (-10.2, 2.2)	-4.5 (-11.2, 2.2)
Physical abilities <sup>a</sup>	Age, gender	99.8 (99.5, 100.3)	0.1 (-1.2, 1.5)	-0.5 (-1.8, 0.8)	-0.6 (-2.0, 0.7)	-2.6 (-4.2, -1.0)**
	Age, gender, 1 BO	98.6 (97.1, 100.1)	0.2 (-1.3, 1.8)	-0.4 (-1.9, 1.0)	-0.6 (-2.1, 0.9)	-1.8 (-3.4, -0.1)*
Bodily pain/ Discomfort	Age, gender	79.9 (74.5, 85.2)	-4.0 (-12.5, 4.5)	-8.3 (-16.2, -0.4)*	-6.3 (-14.5, 1.9)	-9.5 (-18.2, -0.8)*
	Age, gender, 1 BO	74.6 (65.8, 83.4)	-2.7 (-11.9, 6.4)	-7.0 (-15.3, 1.3)	-4.3 (-12.9, 4.4)	-5.0 (-14.3, 4.2)
General Health	Age, gender	75.3 (70.5, 80.1)	-5.0 (-11.7, 1.8)	-6.6 (-12.8, -0.3)*	-7.0 (-13.6, -0.4)*	-9.9 (-16.9, -3.0)**
	Age, gender, 1 BO	76.0 (69.0, 83.0)	-4.8 (-12.1, 2.4)	-5.5 (-12.1, 1.2)	-7.4 (-14.4, -0.4)*	-8.6 (-16.1, -1.1)*
Change in Health	Age, gender	73.1 (63.0, 83.1)	-12.1 (-26.1, 1.9)	-12.5 (-25.9, 0.9)	-8.6 (-22.3, 5.2)	-25.7 (-43.3, -8.2)**
	Age, gender, 1 BO	81.1 (64.4, 97.8)	-16.5 (-31.7, -1.3)*	-14.5 (-28.6, -0.5)*	-13.4 (-28.4, 0.6)	-27.8 (-46.4, -9.1)**

\*p<0.05 \*\*p<0.01 \*\*\*p<0.001 \*\*\*\*p<0.0001

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<sup>a</sup>Interaction with prematurity. Including this variable would have made the regression coefficient for the 5<sup>th</sup> quintile 15.3% more negative.



**Table 3:** Significant associations between severity of acute bronchiolitis, given as need for supportive treatment (oxygen or nasogastric tube feeding), with no support as reference (95% CI), and quality of life domains<sup>abc</sup>, adjusted. Only domains with  $p < 0.05$  included.

QoL domain	Adjustments	No supportive treatment n= 104	Supportive treatment (nasogastric tube or O <sub>2</sub> ) n= 91	Ventilatory support n= 14
Physical abilities <sup>a</sup>	Age, gender	99.7 (99.4, 100.0)	-0.4 (-1.1, 0.3)	-7.3 (-8.9, -5.7)****
General health	Age, gender, 1 BO	98.9 (97.8, 99.9)	-0.6 (-1.5, 0.3)	-6.2 (-8.1, -4.3)****
	Age, gender	75.7 (71.2, 80.1)	-6.0 (-10.1, -1.9)**	-16.1 (-24.6, -7.5)****
	Age, gender, 1 BO	73.9 (68.9, 78.9)	-5.3 (-9.7, -0.9)*	-15.8 (-24.5, -7.0)****
Parental impact – emotions	Age, gender	93.3 (91.9, 94.8)	0.1 (I-2.1, 2.2)	-5.2 (-9.5, -0.9)*
	Age, gender, 1 BO	93.9 (91.4, 96.4)	0.0 (-2.2, 2.2)	-5.2 (-9.4, -1.0)*
Parental impact – time <sup>b</sup>	Age, gender	94.2 (92.4, 95.9)	0.3 (-2.1, 2.8)	-6.5 (-11.7, -1.2)*
	Age, gender, 1 BO	94.0 (90.9, 97.1)	0.4 (-2.3, 3.1)	-6.5 (-11.9, -1.0)*

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\*0.01 ≤  $p < 0.05$  \*\*0.001 ≤  $p < 0.01$  \*\*\*0.0001 ≤  $p < 0.001$  \*\*\*\* $p < 0.0001$

<sup>a</sup>Including prematurity in the model would have made the regression coefficient for ventilatory support 10.5% less negative.

<sup>b</sup>Including prematurity in the model would have made the regression coefficient for ventilatory support 8.9% more negative.

The only QoL domain significantly associated with inclusion severity score was Bodily pain/discomfort, showing a reduction of 4.3 (95% CI 1.9-6.8) % points per point increase of inclusion severity score ( $p=0.0007$ ) after adjustment for age, gender and maternal ethnicity. Oxygen saturation was only associated with Physical abilities, showing a mean age and gender adjusted reduction of 6.7 (95% CI 4.8-8.5)% points in children with SpO<sub>2</sub> lower than 92%. The age and gender adjusted mean score of children with SpO<sub>2</sub> above 92 % at inclusion was 99.8 (95 % CI 99.4 – 100.2),  $p < 0.0001$ .

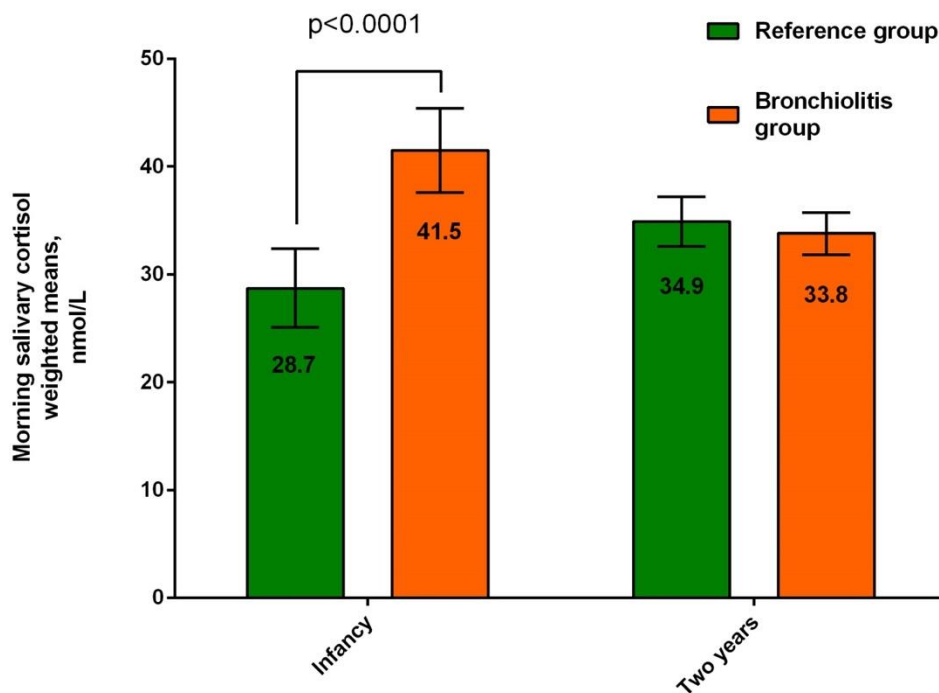
Due to interaction with prematurity for Physical abilities and Parental time, we have also analysed the consequences of stratification of the children into prematurely or full-term born for this domain. Parental time was significantly deteriorated only in the three ventilatory supported prematurely born children, but Physical abilities was worse after ventilatory support both in full-born and prematurely born children. Parental time scores did not differ between prematurely born compared to full term born children without need for supportive therapy. As premature birth in general was not associated with Parental time, we did not include premature birth as a confounder.

Using salivary cortisol as a marker of stress, is stress associated with acute bronchiolitis and/or development of asthma or other atopic diseases? (Papers 3 and 4)

At inclusion, morning salivary cortisol was significantly higher in infants with acute bronchiolitis than in controls, with an unadjusted weighted mean difference of 12.8 (95% CI 7.4, 18.1) nmol/L, (Figure 6) and a weighted mean difference 13.9 (95% CI 8.1, 19.7) nmol/L,  $p < 0.0001$  after adjustment for age and gender.

Among the controls, cortisol at inclusion was not associated with rBO or the number of wheeze episodes at two years of age (results not shown).

**Figure 6**



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Weighted means (95 % CI) of morning salivary cortisol at inclusion and at two years of age.

However, at two years of age, there was no significant difference between the bronchiolitis and control groups, as shown in Figure 5. In contrast to the bronchiolitis group, the morning salivary cortisol in the control (reference) group was significantly higher at two years of age than at inclusion, with a weighted mean difference of 7.8 (95% CI 2.4-13.1) nmol/L,  $p=0.004$ . We found no associations between the first and secondary salivary samples, with a regression coefficient of 0.001 ( $p=0.93$ ) in the control group and -0.010 ( $p=0.83$ ) in the bronchiolitis group. The salivary cortisol values had a higher degree of variation in infancy than at two years of age, as can be found from Tables 4a and 4 b, even if table 4a is split between the groups whereas table 4b is split between the genders.

**Table 4a: 5<sup>th</sup> and 95<sup>th</sup> percentiles of morning salivary cortisol at inclusion. The groups differed significantly and the reference values are based upon the control group. The groups did not differ by gender at inclusion.**

	Reference (Control) group		Bronchiolitis group	
Percentiles	5 <sup>th</sup> (95 % CI)	95 <sup>th</sup> (95 % CI)	5 <sup>th</sup> (95 % CI)	95 <sup>th</sup> (95 % CI)
Inclusion	9.7 (3.1-12.1)	119.6 (64.9-258.6)	12.0 (7.2, 13.4)	112.9 (105.2, 147.0)

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**Table 4b: 5<sup>th</sup> and 95<sup>th</sup> percentiles of morning salivary cortisol at two years. The bronchiolitis and control groups did not differ significantly, but the genders did. Accordingly, reference values at two years are split into genders, but based on both groups.**

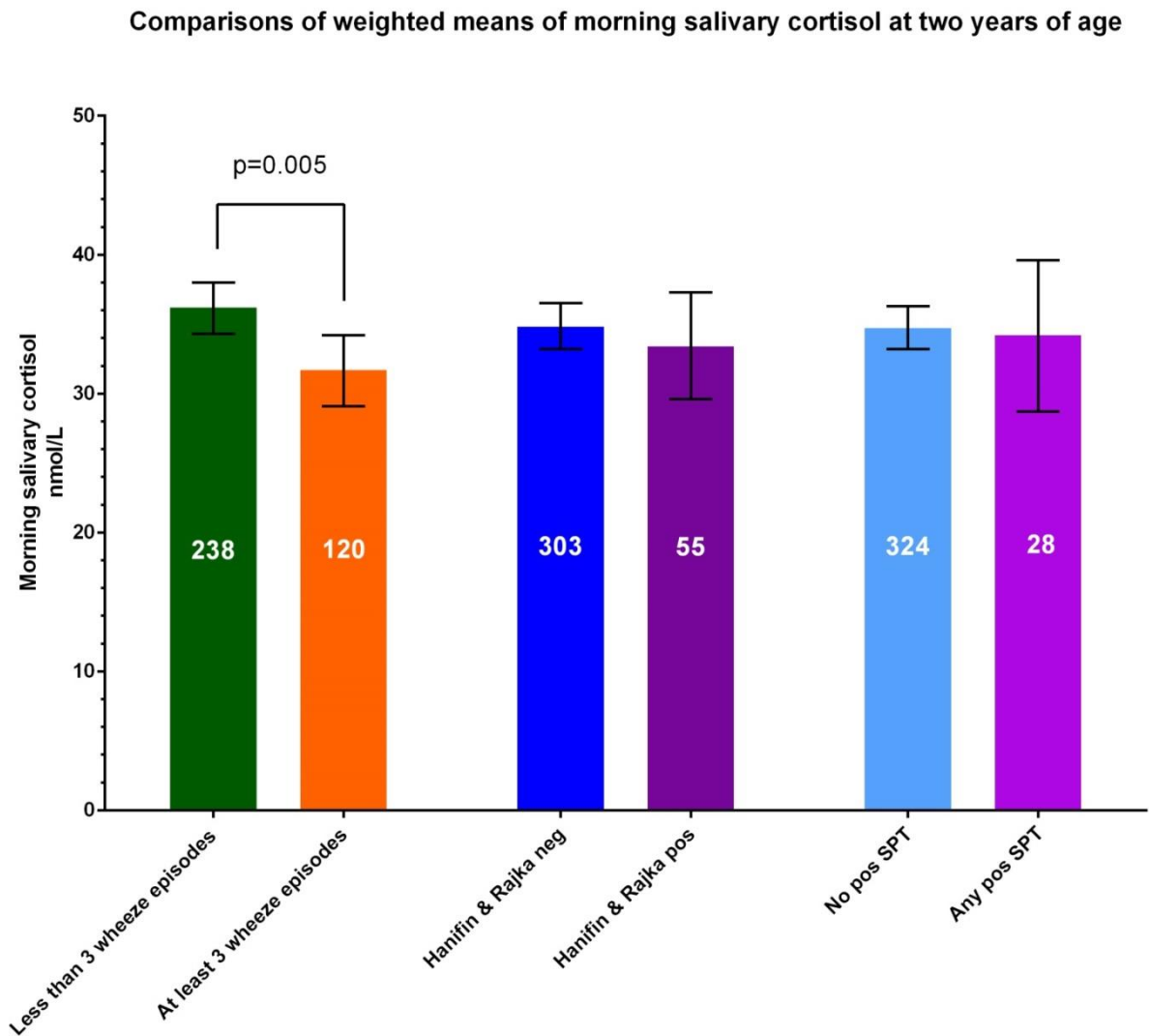
	All boys		All girls	
Percentiles	5 <sup>th</sup> (95 % CI)	95 <sup>th</sup> (95 % CI)	5 <sup>th</sup> (95 % CI)	95 <sup>th</sup> (95 % CI)
Two years	12.7 (8.3, 14.8)	62.8 (55.6, 77.6)	14.4 (12.6, 16.5)	69.7 (62.0, 162.1)

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Salivary cortisol was significantly higher in girls than in boys at two years of age, and data were subsequently adjusted for gender and age. At two years of age, children with rBO had significantly lower morning salivary cortisol than children without rBO, with an age and gender adjusted weighted mean difference of -4.4 (95 % CI -7.4, -1.4) nmol/L.

Salivary cortisol at two years of age was not significantly associated with atopic dermatitis or allergic sensitisation as shown by unadjusted analyses in figure 7.

**Figure 7** Morning salivary cortisol at two years of age, is shown for 358 children included in paper 4, comparing the presence or absence of recurrent bronchial obstruction (rBO), atopic dermatitis (AD) and positive skin prick test (SPT) to common inhalant and food allergens.

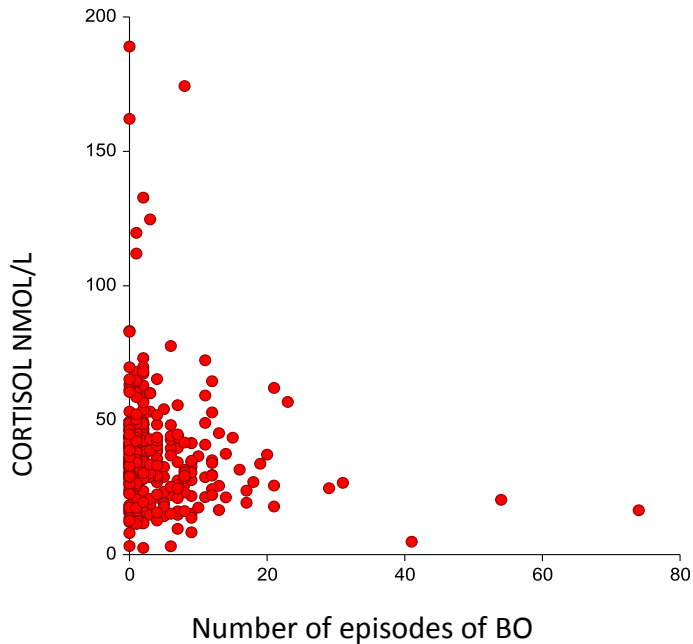


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The number of episodes of bronchial obstruction was significantly inversely associated with salivary cortisol at two years of age (Figure 8).

**Figure 8**

Morning salivary cortisol at two years of age versus the number of parent reported episodes of wheeze in all 379 two-year old children with cortisol results at two years, with or without QoL results



In a regression analysis adjusted for age and gender cortisol decreased by  $-0.3$  nmol/L (95% CI  $-0.5, -0.1$ ),  $p=0.002$  for each increased reported BO episode. We found no significant association between cortisol at two years and the severity of atopic dermatitis by SCORAD index or the number of positive skin prick tests (results not shown).

Is QoL associated with asthma risk factors, asthma and/or other atopic diseases in young children with or without acute bronchiolitis in infancy? (Papers 1 and 4)

The presence of one or more asthma risk factors at inclusion was associated with reduced QoL nine months later as shown in Table 5, with the most pronounced impact observed for the presence of three risk factors.

**Table 5:** The impact on QoL domains by increasing number of the risk factors atopic eczema, parental asthma, parental atopic rhinoconjunctivitis and second-hand smoke as the effect on the change of domain score compared to having no risk factor (0), mean (95% confidence intervals) for children admitted to hospital for acute bronchiolitis before or at inclusion (B) and control children not hospitalised for acute bronchiolitis before inclusion (C). No child had all four risk factors present.

Domain	Group	No risk factor	Change score 1 risk factor	Change score 2 risk factors	Change score 3 risk factors
Overall health	Bronchiolitis	85.7 (82.8, 88.7)	1.1 (-3.2, 5.5)	-1.4 (-6.9, 4.1)	-9.1 (-20.1, 2.0)
	Controls	91.9 (89.0, 94.9)	-0.3 (-4.2, 3.6)	-3.8 (-8.4, 0.8)	-9.4 (-20.8, 2.1)
Physical abilities	Bronchiolitis	99.6 (99.3, 100.0)	0.1 (-0.5, 0.7)	-0.6 (-1.4, 0.3)	<b>-7.1 (-9.1, -5.1)</b> ****
	Controls	100.0 (100.0, 100.0)	0.0 (0.0, 0.0)	<b>-0.3 (-0.7, -0.0)</b> *	-0.1 (-0.6, 0.4)
	Bronchiolitis	94.3 (92.6, 95.9)	-0.8 (-3.1, 1.6)	0.8 (-2.3, 3.8)	-1.5 (-7.7, 4.8)
	Controls	96.1 (94.4, 97.8)	<b>-2.5 (-4.8, -0.2)</b> *	<b>-4.2 (-6.9, -1.5)</b> **	-4.4 (-11.1, 2.2)
	Bronchiolitis	73.5 (69.8, 77.2)	1.4 (-4.0, 6.7)	-5.9 (-12.9, 1.1)	-4.0 (-18.5, 10.4)
discomfort	Controls	76.8 (73.3, 80.3)	-4.7 (-9.5, 0.0)	-2.3 (-7.8, 3.1)	<b>-18.5 (-32.9, -4.0)</b> *
Temperament and moods	Bronchiolitis	83.2 (81.2, 85.2)	1.8 (-1.1, 4.7)	<b>-5.5 (-9.2, -1.7)</b> **	-2.7 (-10.4, 5.0)
	Controls	83.6 (81.9, 85.4)	0.0 (-2.3, 2.4)	-1.4 (-4.1, 1.4)	<b>-10.1 (-17.0, -3.2)</b> **
General behaviour	Bronchiolitis	87.5 (84.8, 90.2)	2.0 (-1.9, 5.9)	-1.4 (-6.2, 3.4)	0.0 (-9.9, 9.9)
	Controls	87.7 (85.0, 90.3)	3.2 (-0.2, 6.6)	0.5 (-3.5, 4.6)	1.9 (-6.8, 10.6)
Overall behaviour	Bronchiolitis	88.0 (85.8, 93.2)	2.0 (-2.7, 6.6)	-1.3 (-7.1, 4.4)	-6.5 (-18.6, 5.6)
	Controls	88.5 (84.6, 92.4)	2.6 (-2.5, 7.6)	-1.5 (-7.6, 4.5)	4.0 (-8.8, 16.9)
Getting along	Bronchiolitis	79.6 (77.3, 81.9)	1.4 (-1.9, 4.7)	-1.1 (-5.3, 3.0)	-0.9 (-9.3, 7.6)
	Controls	77.4 (75.1, 79.8)	1.6 (-1.4, 4.7)	1.1 (-2.5, 4.7)	-1.3 (-9.3, 6.6)
General health	Bronchiolitis	71.1 (68.0, 74.1)	-0.6 (-5.0, 3.7)	-4.7 (-10.4, 1.0)	-2.6 (-14.4, 9.2)
	Controls	84.2 (81.7, 86.7)	-1.8 (-5.2, 1.7)	<b>-5.1 (-9.1, -1.1)</b> *	<b>-11.5 (-21.0, -1.9)</b> *
Bronchiolitis		65.2 (57.9, 72.5)	-2.4 (-12.9, 8.1)	-5.0 (-18.2, 8.3)	-15.2 (-42.8, 12.5)



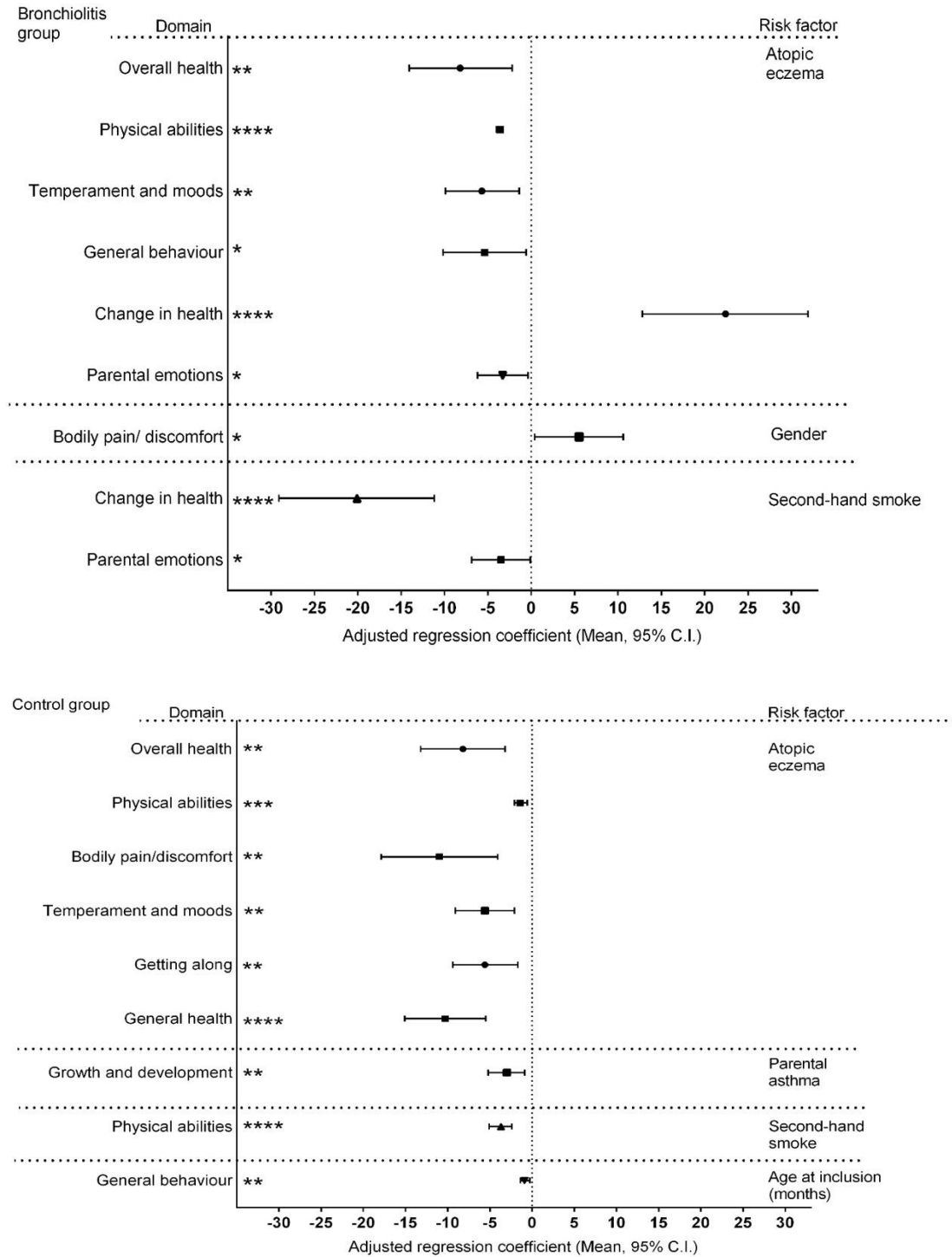
Change in health	Controls	50.0 (50.0, 50.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Parental emotions	Bronchiolitis	93.7 (92.2, 95.1)	1.0 (-1.0, 3.1)	-2.5 (-5.2, 0.2)	<b>-8.0 (-14.4, -1.5)*</b>
	Controls	93.9 (92.3, 95.5)	-0.6 (-2.7, 1.6)	-2.1 (-4.6, 0.5)	-5.4 (-11.4, 0.7)
Parental time	Bronchiolitis	94.3 (92.4, 96.2)	0.1 (-2.6, 2.9)	-1.9 (-5.5, 1.6)	<b>-10.2, -17.8, -2.5)**</b>
	Controls	95.0 (93.1, 96.9)	-1.2 (-3.7, 1.3)	-1.1 (-4.0, 1.8)	-4.5 (-11.6, 2.6)
Family cohesion	Bronchiolitis	85.0 (82.5, 87.5)	0.7 (-2.9, 4.4)	5.3 (0.9, 9.8)	<b>-21.7 (-32.5, -10.9)***</b>
	Controls	85.0 (85.0, 85.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)

\*0.05 > p ≥ 0.01    \*\*0.01 > p ≥ 0.001    \*\*\*0.001 > p ≥ 0.0001    \*\*\*\*p < 0.0001

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Atopic dermatitis was significantly associated with six QoL in both groups (bronchiolitis and controls), second-hand smoking in two and one domain, respectively and parental asthma with one domain among controls only (Figure 9).

**Figure 9** Significant associations between QoL (mean age 14.0 months) and asthma risk factors at enrolment are shown for all 217 children who were previously hospitalised with acute bronchiolitis (top) and 198 controls (bottom) who were not hospitalised for acute bronchiolitis at or before inclusion. The association are given as the regression coefficient (95% confidence interval), adjusted for age and gender in multiple robust regression analyses. Risk factors not shown were excluded by step-down procedure.



\*p<0.05    \*\*p<0.01    \*\*\*p<0.001    \*\*\*\*p <0.0001

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The associations between atopic diseases and QoL at two years of age are shown in Table 6.

In both groups, QoL was negatively influenced by rBO and to a lesser extent by atopic dermatitis. In controls only, QoL was negatively influenced by allergic sensitisation. For some of other domains, associations were weaker, but point in the same direction with negative effects for 9/13 domains in the bronchiolitis group and, positive for Change in health.

Children with compared to without atopic dermatitis had in general more negative QoL, with statistically significant reductions in six domains for all children analysed together.

Allergic sensitisation was significantly associated with reduced QoL in the controls only, but not when all children were analysed together.

**Table 6:** Change of QoL scores (95% CI) at two years of age, age and gender adjusted, by the different expressions of atopic diseases

<b>Recurrent bronchial obstruction</b>			
	<b>Original bronchiolitis group</b>	<b>Controls</b>	<b>Both groups</b>
	<b>N=</b>	<b>N=</b>	<b>N=</b>
OH	-12.3 (-16.5, -8.2)****	-6.6 (-13.9, 0.7)	-11.1 (-14.5, -7.7)****
PA	-1.2 (-2.1, -0.4)**	Not done	Interaction/ Not done
GD	-3.5 (-5.8, -1.2)**	-1.3 (-4.3, 1.8)	-2.7 (-4.4, -1.1)**
BD	-6.5 (-10.8, -2.1)**	-7.3 (-14.7, 0.1)	-5.2 (-8.7, -1.7)**
TM	-3.1 (-5.5, -0.7)*	-4.1 (-7.8, -0.5)*	-2.4 (-4.3, -0.6)**
GB	-4.7 (-7.9, -1.4)**	-0.7 (-5.5, 4.1)	-3.4 (-5.9, -1.0)**
OB	-0.0 (-0.0, 0.0)	Not done	Not done
GA	-1.9 (-4.2, 0.4)	-4.0 (-7.9, -0.0)*	-2.0 (-3.8, -1.0)*
GH	-13.8 (-17.8, -9.8)****	-6.8 (-12.9 -0.7)*	-15.1 (-18.2, -12.0)****
CH	6.9 (0.8, 13.0)*	6.8 (-0.6, 14.2)	11.8 (7.7, 15.9)****
PE	-4.0 (-6.4, -1.5)**	-2.3 (-5.9, 1.4)	-3.0 (-4.9, -1.2)**
PT	-2.5 (-4.5, -0.5)*	-2.1 (-5.0, 1.0)	-1.6 (-3.1, -0.2)*
FC	-0.1 (-4.7, 4.5)	0.3 (-7.4, 8.0)	-0.4 (-4.1, 3.3)
<b>Atopic dermatitis</b>			
	<b>Original bronchiolitis group</b>	<b>Controls</b>	<b>Both groups</b>
OH	-5.5 (-11.7, 0.6)	-6.3 (-13.6, 0.9)	-5.7 (-10.5, -1.0)*
PA	-0.0 (-0.0, 0.0)	-2.1 (-3.2, -1.0)***	-0.0 (-0.1, 0.1)
GD	-2.5 (-5.8, 0.7)	-1.2 (-4.2, 1.8)	-1.9 (-4.1, 0.3)
BD	-7.2 (-13.4, -0.9)*	-1.7 (-8.6, 5.3)	-4.6 (-9.2, 0.1)
TM	-5.1 (-8.4, -1.8)**	1.0 (-2.6, 4.5)	-2.2 (-4.7, 0.2) interaction
GB	-5.3 (-9.9, -0.7)*	-4.1 (-8.8, 0.6)	-4.8 (-8.1, -1.6)**
OB	0.0 (0.0, 0.0)	Not done	Not done
GA	-6.2 (-9.3, -3.0)***	-0.9 (-4.8, 2.9)	-3.8 (-6.2, -1.3)** interaction
GH	-5.8 (-12.0, 0.3)	-7.0 (-13.0, -1.0)*	-6.5 (-11.1, -1.9)**
CH	7.8 (-0.9, 16.4)	3.1 (-3.3, 9.6)	5.6 (0.0, 11.1)*
PE	-5.7 (-9.2, -2.2)**	-1.6 (-5.2, 2.0)	-3.6 (-6.0, -1.1)**
PT	-1.9 (-4.8, 1.0)	-1.9 (-4.9, 1.0)	-2.1 (-4.2, 0.0)
FC	-5.4 (-11.9, 1.1)	0.7 (-6.7, 8.1)	-2.6 (-7.5, -2.3)
<b>Allergic sensitisation</b>			
	<b>Original bronchiolitis group</b>	<b>Controls</b>	<b>Both groups</b>
OH	-0.7 (-8.6, 7.2)	-11.7 (-21.6, -1.9)*	-4.8 (-11.1, 1.4) interaction
PA	0.0 (-0.2, 0.2)	Not done	Not done
GD	-0.1 (-4.3, 4.0)	-1.9 (-6.4, 2.6)	-0.7 (-3.7, 2.3)
BD	4.2 (-3.6, 12.1)	-6.4 (-16.8, 4.0)	1.2 (-5.0, 7.4)
TM	-2.5 (-1.8, 6.8)	-1.3 (-6.5, 3.9)	1.2 (-2.1, 4.5)
GB	-0.8 (-6.8, 5.2)	-7.0 (-13.7, -0.3)*	-3.2 (-7.7, 1.2)
OB	0.0 (-0.0, 0.0)	Not done	Not done
GA	-0.4 (-4.6, 3.8)	-8.0 (-13.6, -2.4)**	-2.9 (-6.3, 0.5) interaction
GH	0.1 (-7.9, 8.1)	-12.2 (-20.5, -3.9)**	-4.9 (-11.1, 1.3)
CH	-1.0 (-12.3, 10.3)	7.3 (-2.8, 17.4)	2.2 (-5.6, 10.0)
PE	-0.2 (-4.7, 4.3)	-5.8 (-10.7, -1.0)*	-2.4 (-5.7, 1.0)
PT	-0.0 (-3.7, 3.7)	-4.3 (-8.3, -0.2)*	-1.6 (-4.4, 1.2)
FC	-0.0 (-3.7, 3.7)	-1.8 (-12.3, 8.7)	-3.0 (-9.5, 3.5)

Not done refers to analyses that were not applicable due to strong correlations between some of the included variables.

OH = Overall health; PA = Physical abilities; GD = Growth and development; BD = Bodily pain/ discomfort; TM = Temperament and moods; GB = General behaviour; OB = Overall behaviour; GA = Getting along; GH = General health; CH = Change in health; PE = Parental impact – emotions; PT = Parental impact – time; FC = Family cohesion

\*p<0.05 \*\*p<0.01 \*\*\*p<0.001 \*p<0.0001

In sensitivity analyses using quantitative exposures, data are reported stratified for the two groups due to significant interactions between enrolment group (acute bronchiolitis or controls) for Physical abilities and Bodily pain/ discomfort for the total number of wheeze episodes, for General behaviour for SCORAD and for General health for the sum of positive skin prick test diameters. The associations were generally in accordance with the categorical measures rBO, AD and allergic sensitisation. The number of wheeze episodes was negatively associated with nine and five domains in the bronchiolitis group and control groups, respectively. A difference between the 95- and 5-percentiles of the number of wheeze episodes for the bronchiolitis group for the most negatively affected domains represents a reduction of Overall health, Bodily pain/ discomfort and General health domains of 6.8, 8.5 and 11.9 percentage points, respectively. Corresponding results for the control group are 10.2, 9.6 and 10.9 percentage points.

The SCORAD was negatively associated with seven and two domains in the bronchiolitis and control groups, respectively. A difference between the 95- and 5-percentiles of SCORAD points for the bronchiolitis group represents a reduction of Overall health, Bodily pain/ discomfort and General health domains of 8.7, 11.6 and 8.7 percentage points, respectively. Corresponding results for the two associated domains of the control group are 11.1, and 7.4 percentage points for the Overall health and General health domains, respectively.

The sum of positive skin prick test diameters was negatively associated with zero and four domains in the bronchiolitis and control groups, respectively.

Pre-defined clinically relevant differences were observed within the domain Overall health and General health with significantly lower scores for children with rBO, whereas the reduced QoL by other asthma risk factors did not reach the pre-defined clinical relevance level, although some were statistically significantly reduced (Table 6).

Is there a link between QoL, salivary morning cortisol and atopic diseases, and if so, will such a link be modified by acute moderate to severe bronchiolitis in infancy? (Paper 4)

#### Cortisol at inclusion and QoL nine months after inclusion

In the bronchiolitis group we found an age- and gender adjusted negative association between cortisol at inclusion and QoL nine months later for the domain Overall behaviour, only. The change in QoL by -0.14 percentage points (95% CI -0.22, -0.06) per nmol/L increase in cortisol ( $p=0.0008$ ) corresponded to a difference of -15.4 percentage points between subjects with morning salivary cortisol at the 95<sup>th</sup> and 5<sup>th</sup> percentiles.

Likewise, in the control group a significant positive age- and gender adjusted association was observed between cortisol at inclusion and QoL for the domain Change in health by 0.06 percentage points (95% CI 0.04, 0.09) per nmol/L increase in cortisol ( $p<0.0001$ ), corresponding to a 6.6 percentage points difference between subjects with cortisol at the 95<sup>th</sup> and 5<sup>th</sup> percentiles.

#### Cortisol at inclusion and QoL at two years of age

In the bronchiolitis group, no associations were found between cortisol at inclusion and QoL at two years of age. In the control (reference) group, cortisol at inclusion was associated with Change in health reported at two years. Adjusted for age at inclusion and gender, the score increased by 0.05 (95% CI 0.02, 0.08) percentage points for each nmol/L change of cortisol

( $p=0.0004$ ). This corresponded to a difference in cortisol of 5.5 percentage points between subjects with cortisol levels at the 95- and 5-percentiles points. (Not previously published).

QoL nine months after inclusion and cortisol at two years of age

The groups were stratified due to significant interactions between cortisol and group affiliation for Overall health and Temperament and moods.

In the bronchiolitis group, but not the control group, age and gender adjusted analysis QoL<sub>1</sub> scores (mean age 14.0 months) were significantly and positively associated with cortisol at two years of age in for eight domains. In the group of children with QoL assessments at both time points, the associations were significant for seven domains at two years, as shown in paper 4 and in Table 7. The maximal relative influence on the associations between QoL and cortisol by atopic manifestations was seen for Parental impact – emotions by the presence of all three atopic manifestations with 16.7% reduction of the associations.



**Table 7:** Modification of associations of morning salivary cortisol at two years of age and QoL nine months after inclusion, at a mean age of 14 months<sup>1</sup> by atopic diseases, bronchiolitis group, is shown by per cent changes in the associations between salivary morning cortisol and QoL, adjusted for age and gender (left column). The per cent difference refers to change in salivary cortisol, nmol/L, per QoL score unit (95% CI)

Change in cortisol nmol/L per QoL score change (95% CI)		rBO <sup>2</sup>	AD <sup>3</sup>	Allergic sensitisation <sup>3</sup>	All three allergic diseases
		Percentage change by atopic manifestations			
Overall health	0.2 (0.0, 0.3) <sup>*</sup>	-7.6	-8.4	4.5	-12.4
Physical abilities	0.9 (0.4, 1.4) <sup>***</sup>	-2.5	-4.9	2.6	-6.0
Growth and development	0.3 (0.1, 0.6) <sup>**</sup>	-3.2	-6.9	1.2	-11.7
Temperament and moods	0.4 (0.1, 0.6) <sup>**</sup>	-2.5	-12.6	8.1	-6.7
General health	0.2 (0.0, 0.3) <sup>*</sup>	-9.3 <sup>2</sup>	-9.5 <sup>2</sup>	8.8	-11.8
Parental impact - emotions	0.5 (0.2, 0.7) <sup>***</sup>	-4.7	-10.7	-0.2	-16.7
Parental impact - time	0.3 (0.1, 0.5) <sup>**</sup>	-4.8	-8.8	5.7	-9.0

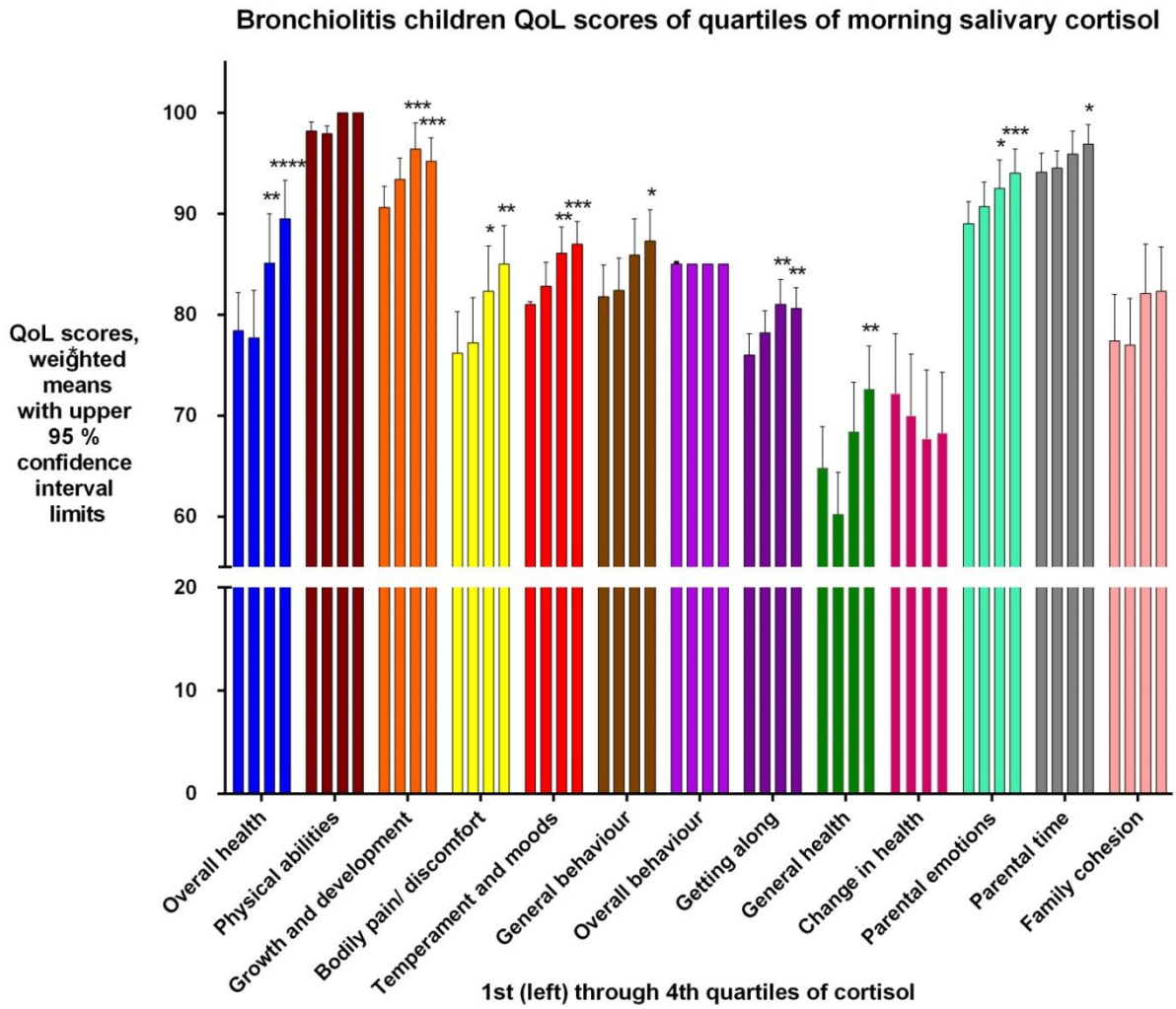
<sup>1</sup>At the first survey, the domains General behaviour, Overall behaviour, Getting along and Change health were not scored for children younger than 12 months.

<sup>2</sup>Changing the p-values to >0.05

Cortisol at two years of age and QoL at two years of age.

Due to interaction between affiliation group and cortisol for five domains; Overall health, Growth and development, Temperament and moods, General behaviour and Parental impact – emotions, we stratified the analyses between the original bronchiolitis and control groups. In the bronchiolitis group, significant associations were seen in 9/13 domains by unadjusted analyses, , with QoL increasing with increasing cortisol, see Figure 10, but 8/13 domains after adjustment for age and gender for 8/13 domains, see Table 8. In the control group, we found a change of 0.1 (95% CI 0.0, 0.2) percentage points of General health per nmol/L change of salivary cortisol ( $p=0.046$ ) after adjustment for age and gender.

Figure 10



\* p<0.05 \*\* p<0.01 \*\*\* p<0.001 \*\*\*\*p<0.0001

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**Table 8: Change of QoL at two years of age, change (95 % CI), percentage points, per nmol/L difference in cortisol, age and gender adjusted**

<b>Domain</b>	<b>Bronchiolitis group</b>	<b>Control group</b>	<b>Both groups</b>
Overall health boys	0.31 (0.17, 0.45)****	0.00 (-0.19, 0.19)	Interaction
Overall health girls	-0.00 (-0.16, 0.16)	0.01 (-0.12, 0.15)	Interaction
Overall health total	0.19 (0.07, 0.30)**	0.01 (-0.10, 0.12)	Interaction
Physical abilities	0.00 (0.00, 0.20)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
Growth and development	0.07 (0.02,0.13)**	-0.00 (-0.05, 0.04)	Interaction
Bodily pain/ discomfort	0.12 (0.01, 0.23)*	0.05 (-0.06, 0.16)	0.08 (0.00, 0.16)*
Temperament and moods	0.12 (0.06, 0.18)**	-0.03 (-0.09, 0.02)	Interaction
General behaviour	0.09 (0.01, 0.17)*	-0.02 (-0.10, 0.06)	Interaction
Overall behaviour	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
Getting along	0.08 (0.02, 0.13)**	-0.00 (-0.06, 0.06)	0.04 (0.00, 0.08)*
General health	0.11 (-0.00, 0.22)	0.10 (0.00, 0.19)*	0.12 (0.04, 0.20)**
Change in health	0.03 (-0.12, 0.19)	0.01 (-0.11, 0.09)	0.02 (-0.11, 0.08)
Parental impact – emotions	0.09 (0.03, 0.15)**	-0.02 (-0.08, 0.03)	Interaction
Parental impact – time	0.06 (0.01, 0.10)*	0.01 (-0.04, 0.06)	0.04 (0.00, 0.07)*
Family cohesion	0.09 (-0.02, 0.21)	-0.04 (-0.16, 0.08)	0.03 (-0.05, 0.11)

The effect size (change in percentage point) on QoL score per nmol/L cortisol decreased for five domains when both groups were analysed together for domains without interaction with affiliation group, compared to analysis of the bronchiolitis group. For two of the three remaining domains, Physical abilities and Overall behaviour, robust regression was not reliable due to low variation of the single data.

We did not find any significant changes by adjusting for socio-economic factors.

For the bronchiolitis group, adjusted analyses showed that Overall health in boys and General health (in all) were influenced by rBO mostly, while atopic dermatitis only weakly influenced

the associations, and allergic sensitisation did not influence the associations between cortisol and QoL see Table 9.

**Table 9:** The potential influence of recurrent bronchial obstruction (rBO), atopic dermatitis (AD) and allergic sensitisation (AS) on the associations between Quality of Life (QoL<sub>2</sub>) and salivary cortisol at two years of age is shown for 203 children who were included when they had moderate to severe acute bronchiolitis in infancy. Only domains with changes with p-values maximally 0.05 included.

The influence by including each atopic manifestation (rBO, AD and AS) is shown as the percentage change of QoL per 1 nmol/L change in salivary cortisol, adjusted for age and gender. Each column includes all children with the observed atopic manifestation, and they are not mutually exclusive.

Domain (Mean domain score difference <sup>1</sup> by difference between 95 <sup>th</sup> and 5 <sup>th</sup> percentile of cortisol, 51.6 nmol/L)	Change in QoL <sub>2</sub> score per nmol/L unit salivary cortisol	rBO	AD	Allergic sensitisation	All three atopies <sup>1</sup>
Overall health boys (16.0) <sup>2</sup>	0.31 (0.17, 0.45)****	-20.7	-2.1	-0.5	-20.1
Overall health girls (-0.0)	-0.00 (-0.16, 0.16)				
Growth and development (3.8)	0.07 (0.02, 0.13)**	-1.4	1.7	0.6	-0.9
Bodily pain/ discomfort (6.2)	0.12 (0.01, 0.23)*	-8.3	5.4	-2.3	-8.8
Temperament and moods (6.1)	0.12 (0.06, 0.18)***	-6.9	1.4	-1.5	-8.2
General behaviour (4.6)	0.09 (0.01, 0.17)*	-6.7	-1.5	1.3	-6.2
Getting along (4.0)	0.08 (0.02, 0.13)**	-3.0	-0.3	0.9	-4.3
General health (5.6)	0.11 (-0.00, 0.22) <sup>3</sup>	-26.9	0.4	0.3	-32.9
Parental impact –Emotions (4.5)	0.09 (0.03, 0.15)**	-6.1	-5.1	1.2	-12.5
Parental impact – Time (3.0)	0.06 (0.01, 0.10)*	-7.7	-0.1	0.9	-5.3

<sup>1</sup>The change by introducing all three atopic manifestations into the regression model, not restricted to subjects with all three diseases

<sup>2</sup>Stratified into genders due to interaction

<sup>3</sup>p=0.0517. \*p<0.05 \*\*p<0.01 \*\*\* p<0.001 \*\*\*\*p<0.0001

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We also estimated the influence of the degree of atopic disease by adjustment of the continuous variables total no. of wheeze episodes; both the total no. of wheeze episodes and SCORAD; and by the total no. of wheeze episodes, SCORAD and sum of skin prick test wheal diameters after adjustment for age and gender for the bronchiolitis group. The association between cortisol and General health was reduced by more than 25% by introducing the number of wheeze episodes into the model. We also found that the number of wheeze episodes and SCORAD, and the number of wheeze episodes, SCORAD and allergic sensitisation reduced the associations by more than 25% for Overall health. Introducing the severity variables of all the atopic diseases turned the associations insignificant for Bodily pain/ discomfort, General behaviour and General health, and the same result was seen by only including the number of wheeze episodes or the number of wheezing episodes and SCORAD. Introducing only SCORAD or only the sum of skin prick test wheal diameters did not change the number of domains being significantly associated with cortisol. Details are shown in Table 10.

**Table 10: Per cent change of the association between QoL and morning salivary cortisol, both at two years of age, by introducing into the model the total number of wheeze episodes, SCORAD index and the sum of positive skin prick test wheal diameters in addition to age and gender, bronchiolitis group.**

<b>Domain</b>	<b>Modification by no. of wheeze</b>	<b>Modification by no. of wheeze and SCORAD</b>	<b>Modification by no. of wheeze, SCORAD and sum of SPT</b>
Overall health	-22.6%	-26.5%	-25.4%
Growth and development	-16.2%	-14.2%	-14.9%
Bodily Pain/ Discomfort	-24.5% <sup>1</sup>	-20.0% <sup>1</sup>	-21.6% <sup>1</sup>
Temperament and moods	-15.6%	-13.2%	-12.9%
General behaviour	-13.1% <sup>1</sup>	-13.6% <sup>1</sup>	-14.1% <sup>1</sup>
Getting along	-7.8%	-7.5%	-7.7%
General health	-37.4% <sup>1</sup>	-38.8% <sup>1</sup>	-39.0% <sup>1</sup>
Parental Impact – Emotions	-17.7%	-15.1%	-16.1%
Parental impact - time	-15.5%	-14.7%	-11.6%

<sup>1</sup>With the included variables in the models, p-values were >0.05 for the marked results.

Due to an interaction between cortisol and gender for Overall health, we gender stratified the analysis for association between Overall health and cortisol as shown in Table 9. This was the only domain with a gender interaction between QoL and cortisol. If we include children from the population based control group who had been hospitalised for acute bronchiolitis before inclusion, the number of subjects to be analysed increased from 203 to 206. The number of domains with significant associations between cortisol and QoL after adjustment for age and gender increased to nine, also including General health. Details are not shown.



In order to further investigate if a higher rate of asthma in the bronchiolitis group may explain why we find associations between cortisol and QoL only in the bronchiolitis group, we have investigated results in children without rBO. For seven domains in the bronchiolitis group, there was still an association between cortisol and QoL (details not shown). To investigate if QoL is associated with cortisol in children with rBO both in the bronchiolitis and control groups, we analysed both groups together in children with at least three wheeze episodes for all domains, as interaction with group affiliation was not found for children with rBO. The power was low, and associations were found only for two domains (details not shown). Even when we studied cortisol as a dependent variable of the 5-scale Change in health score as a categorical variable as another method of robust regression, we did not find any significant results in any of the groups for the association between cortisol and Change in health. We found no interaction between cortisol and group affiliation for this domain, so we also tried to analyse the groups together, finding a negative association between cortisol and Change in health (results not shown).

## DISCUSSION

Is acute bronchiolitis in infancy or the severity of the disease associated with reduced QoL in young children?

Infants with acute bronchiolitis had similar reductions in QoL both nine months after inclusion and at two years of age compared to controls. Disease severity was associated with the reduction in QoL nine months later.

Our study demonstrated variations in associations between disease and the different QoL domains. Reductions in the domain Physical abilities were associated with different respiratory disease characteristics. Low SpO<sub>2</sub> at inclusion also had a negative impact on this domain. Similarly, a study of persistently wheezing four-year-old children showed an association between a physical summary score of the Child Health Questionnaire CHQ PF-28 and wheezing (91). We adjusted for age, to avoid any possible confounding by age on the results.

The reductions of Overall health and General health after acute bronchiolitis seem to be of at least moderate degree, comparing with estimates by the Generation R study (50). The reduced QoL<sub>1</sub> observed for the domains Overall health and General health and increased for Change in health after acute bronchiolitis at both time points are in line with a previous report in children with the chronic disease neurofibromatosis type 1 and after RSV bronchiolitis (41), in pre-school children with wheezing illness (83) and with functional abdominal pain (92). In children with functional abdominal pain, the scores of Change in health were better irrespectively of the duration of the pain, indicating that reporting a positive change in health is not necessarily associated with rapid improvement from disease symptoms, but rather characteristic of a population with a chronic disease.

The inverse association between severity of bronchiolitis in infancy and later QoL in our studies are partly novel, as we are unaware of studies reporting associations between severity

characteristics such as LOS, need for nasogastric feeding or ventilatory support. However, Bont et al. found no significant associations between QoL and mechanical ventilation in 28 of 128 infants hospitalised with RSV infections, but used another questionnaire assessing 13 scales including stomach, skin, appetite and liveliness, and the measurement took place at three years of age (93).

The negative impact by supportive treatment other than ventilatory support on QoL<sub>1</sub> was similar to that of atopic dermatitis. The reductions of scores nine months after inclusion in the four domains Physical abilities, General health, Parental impact – emotions and Parental impact – time by ventilatory support, in practice continuous proximal airway pressure (CPAP) have, to the best of our knowledge, not been demonstrated before.

The 52 % rate of QoL form response in the bronchiolitis group may be of limited bias as the respondent and non-respondent groups were similar in most background characteristics.

However, the respondents and non-respondent groups differed by parental education and ethnicity. This way, the respondents of the bronchiolitis group differed less from the control group than the non-respondents. These factors were not significant confounders and did not interact with our analyses.

One may ask if prematurity could be a hidden explanation of the associations between acute bronchiolitis and QoL, but testing confounding by introducing prematurity into the models did not significantly change the results.

**Is stress as measured by morning salivary cortisol associated with acute bronchiolitis and with development of asthma or other atopic diseases?**

Morning salivary cortisol was higher among children with acute bronchiolitis compared to controls at inclusion, but not at two years of age. The variation in salivary cortisol was greater at inclusion than at two years of age, when values were higher in girls than boys and lower

among children with rBO. Salivary values were higher at two years compared to inclusion in the control group, only.

Our finding that cortisol in infants with acute bronchiolitis was higher than among controls, but not at two years of age indicates possible pathophysiological involvement of cortisol in acute bronchiolitis. This is in line with higher cortisol values in severe than mild disease of RSV infection in infants (94).

Although there was no significant difference between the groups' morning salivary cortisol values at two years of age, the lower cortisol in subjects with rBO and the negative association between morning salivary cortisol and the total number of reported wheeze episodes in the bronchiolitis group and both groups together, with a lower p-value when both groups are included, is a strong indicator of an important role of cortisol in the development of asthma in early childhood.

The observed reduced cortisol levels at two years in children with rBO is supported by studies showing reduced baseline, i.e. unstimulated cortisol in older children with asthma (74), as well as supported by the flattened diurnal cortisol variation, with lower morning salivary cortisol in infants with asthmatic or allergic mothers reported by Ball et al (73). In line with our findings, L. Dreger et al. found that maternal stress beyond the neonatal period was associated with a 5.2 % decrease in serum cortisol in 7 to 10 year old children with asthma as opposed to a 25.9 % increase in cortisol in children without asthma (3).

The lack of association between cortisol and atopic dermatitis is in contrast to the findings by F. Stenius et al. who reported higher morning salivary cortisol in two-year-old children with atopic dermatitis, they found a tendency to lower cortisol in children with recurrent wheeze (76). On the other hand, R. Kojima et al. found a blunted salivary cortisol response to acute stress in children with a median age of 16.5 months, dependent of the severity of atopic

dermatitis (75). The lack of associations between atopic dermatitis and morning salivary cortisol among our children may be due to the mild disease in most children, as few of them had severe atopic dermatitis.

Our study was not designed to answer a question about a possible reverse causation; that children with a low cortisol response or a low morning salivary cortisol were more prone to development of atopic disease, rather than developing a low morning salivary cortisol due to development of atopic disease in the first place. As we have shown, the morning salivary cortisol values taken at inclusion had a higher variability than the two-year-of-age samples. Although acute bronchiolitis can be attributed to acute stress, an association with future atopic disease by cortisol at inclusion is not a subject of this study. The cortisol samples taken at two years of age were taken in the homes, i.e. the children were not hospitalised due to any acute disease. On the other hand, the procedure of taking a salivary sample as soon as possible, before the first meal after 6:00 a.m. can possibly catch a cortisol awakening response in some children, reflecting a capability for reacting with a rise of cortisol in acute stress in addition to the natural circadian rhythm with higher salivary cortisol values in the morning, both being expressions of the adrenal glands' cortisol production capability.

**Is QoL associated with asthma or asthma development, risk factors for asthma and other atopic diseases in young children, with or without acute bronchiolitis in infancy?**

The associations differed between the groups. In the bronchiolitis group, QoL<sub>2</sub> was significantly negatively associated with rBO and AD, but among the controls, QoL<sub>2</sub> was negatively associated with the severity of AD and sum of skin prick test wheal diameters. However, the number of subjects with rBO in the control group was small, and the effect sizes of the number of wheeze episodes in the groups were similar. Accordingly, it seems that the degree of wheeze has a similar effect on QoL<sub>2</sub> in both groups. QoL<sub>1</sub> was especially negatively associated with atopic dermatitis, less with other risk factors and not with parental allergic

rhinitis. Our finding of a stronger association between parent reported diagnosis of atopic dermatitis in the first year of life and reduced QoL than our diagnosis of atopic dermatitis at two years of age and concomitantly reduced QoL may be partially explained by the selection of parents reporting doctor diagnosis of atopic dermatitis, indicating that the children had been more severely affected if the parents found it necessary to contact a doctor. However, our finding is also in accordance with L. Rystedt's finding that early onset of atopic dermatitis is associated with a poorer prognosis (95).

Our findings are in accordance with a previous study of a negative association of QoL as measured by ITQOL with asthma-like symptoms in the first year of life (50). However, except for atopic dermatitis in infancy, to the best of our knowledge, a relationship between with asthma risk factors in infancy and QoL in the first two years of life has not been studied before. Adjustment for multiple outcomes was not done, but was not appropriate, as the outcome variables, the ITQOL domains, were not independent from each other.

**Is there a link between QoL, morning salivary cortisol and atopic diseases that is influenced by moderate to severe acute bronchiolitis in infancy?**

We found that QoL was linked to morning salivary cortisol at two years of age in the bronchiolitis group, but not in the control group after adjustment for age and gender. This link could be partly explained by recurrent bronchial obstruction, but to a little extent by atopic dermatitis. Our finding is in accordance with E. Bakkeheim et al.'s finding of a lower morning salivary cortisol in subjects with asthma (74). It is also in accordance with T. Ball et al.'s finding of a flattened diurnal cortisol variation in infants with asthmatic or allergic asthma (73).

The differences between the groups could not be explained merely by a higher rate of recurrent bronchial obstruction in the bronchiolitis group, according to analyses both groups in subjects with recurrent bronchial obstruction of domains without interaction between the

affiliation groups. It is likely that the less obvious impact of atopic dermatitis on the associations between morning salivary cortisol and QoL in our children can be attributed to the fact that most of the children did not have severe atopic dermatitis. The differences between the groups could not be completely explained by socio-economic differences, as adjustment for these differences did not change the results significantly. It may seem contradictory that we found that acute bronchiolitis at inclusion was associated with high morning salivary cortisol, but that chronic stress as measured by a low QoL in these individuals lead to a lower response to the acute stress or adrenal gland response to the process of becoming awake in the morning and circadian rhythm at two years of age. However, if we hypothesise that the process of mobilising the adrenal cortex to increase cortisol secretion in the late night and early morning hours before awakening is similar to the adrenal glands' response to acute stress, our study is a contribution to a hypothesis that in children developing asthma, e.g. in children who had moderate to severe acute bronchiolitis in infancy, the body is changed in a direction similarly to subjects with asthma when it comes to the later reduced cortisol response in acute stress in individuals exposed to chronic stress.

Our study was not designed to distinguish between a lower morning salivary cortisol due to a lower capability to respond to acute stress in atopic children exposed to chronic stress or a lower capability for a morning surge of cortisol due to asthma. However, both the study showing a lower capability for a cortisol surge by acute stress in atopic children exposed to maternal or chronic stress (3) and Bakkeheim's finding of a lower morning cortisol in children with asthma and allergic rhinitis support our findings, as children who have undergone acute bronchiolitis in infancy are prone to develop asthma. Our adjustment for recurrent bronchial obstruction as a proxy for asthma, showing that significant associations between morning salivary cortisol and QoL persist, indicate that associations are found not only for children with asthma, but for children at risk for asthma.

The greater variability in morning salivary cortisol at inclusion could explain why we found no association with QoL nine months later except for Overall behaviour in the bronchiolitis group and Change in health in the control group. These findings, being solitary, may be casual, especially for Overall behaviour, showing low variability and no associations in other contexts.

The finding of a negative association between Change in health at two years of age with cortisol for both groups together is in line with the trend as shown for Change in health opposed to other domains, indicating chronic disease.

## Strengths and limitations

### Study design

The prospective design of the study and follow-up of the children until two years of age are among the strengths. A reasonably large group of children were included in infancy, at an early stage of any possible atopic disease. Although atopic disease was not an inclusion criterion, a reasonably large group of children showed signs of atopic disease. Although objective signs of atopic dermatitis were not scored objectively at inclusion, the signs of atopic disease before inclusion were recorded by interviews of the parents. At two years of age, signs of asthma were estimated by summing the number of wheeze episodes reported by the parents at inclusion and consecutive episodes at the two-year-of-age visit. At this visit, atopic dermatitis was diagnosed by examination, and allergic sensitisation by skin prick testing.

One limitation could be the absence of diary based ratings of symptoms of wheeze, atopic dermatitis or allergic sensitisation. On the other hand, daily time consuming procedures from early infancy until two years of age would increase the likelihood of drop-outs or lower numbers of participating children, especially in the bronchiolitis group, when parents had to



decide quickly whether or not they would participate, in a situation when their child were hospitalised for a possibly serious acute disease.

One limitation is the lack of QoL scoring at inclusion, at the same time as the first cortisol measurement. On the other hand, by scoring QoL nine months after the episode of acute bronchiolitis QoL is likely to reflect a longer lasting trend, less influenced by temporary circumstances at the acute episode.

## Concepts

### ***Acute bronchiolitis***

We used the bronchiolitis severity criteria introduced by Kristjansson et al., well known by our research group from another Scandinavian study (80). This method was found better than another method for severity scoring by H.J. Chin and Q.B. Seng (96). By using strict criteria of acute bronchiolitis at inclusion, excluding mild bronchiolitis, we increased the likelihood that the children really had acute bronchiolitis. Similarly, this way, the risk of disturbance of other factors on the reasons for hospitalisation or longer hospital stays, e.g. social factors, were reduced. The risk of confusion with asthma or other chronic lung disease was reduced by the upper age limit of 12 months and excluding children with more than one episode of wheeze or more than one month of daily cough, or children with other chronic disease that may interfere with the results. One limitation is that Kristiansson's severity score has not been included among the best six of 32 recently listed methods of severity descriptions by C. Rodriguez-Martinez et al. (97), but it has close similarities with one of the top six methods; Wood Downes's modified by Ferres score; containing rates of cyanosis (we rated the colour); ventilation (restriction in range; not relevant in our study including infants with a maximum of one episode of bronchoconstriction before inclusion and exclusion of subjects with any chronic disease that may significantly influence the clinical course of acute bronchiolitis); wheezing (we rated lung auscultation); retraction (i.e. inspiratory recessions rated by us);

respiratory rate (also included in our score) and heart rate (not rated by us, but will probably influence the child's general condition (rated by us). Besides, the main purpose of our scoring was to distinguish between children with only mild disease and those with moderate or severe bronchiolitis, not selecting children who needed hospital in-patient treatment. Our efforts to distinguish bronchiolitis from asthma and selection of only moderate to severe infants, makes it unlikely that confusion with other diseases is a major confounder.

### ***Health-related quality of life in young children***

The reliability and validity of the Infant and Toddler Quality of Life Questionnaire has been shown by H. Raat et al. (51). The full-length version, ITQOL-97, has been validated into Norwegian by a rigorous procedure involving translation by two in-country experts and back-translation by a native English speaker who is bilingual in the target language, and debriefing interviews with parents.

One may question why the variability is so low for the domains Physical abilities at two years of age. For parents with two-year-old children, it seems that this domain has a low sensitivity for the total score. Even if the parents are asked to take the children's age into consideration, for most two-year-old children, questions about feeding, grasping, reaching, rolling over and crawling are likely to be answered with full scores. The last question is about running, which may be more relevant for a two year old child, but the total score for two year old children has a high number of maximal scores, i.e., a ceiling phenomenon is a greater problem for this domain than for other domains at two years of age.

Another domain with low variation of scores is Overall behaviour. Parents are asked how they would rate their child's behaviour overall, i.e. with one question, comparing their child with other children at the same age. Among the five answering alternatives, the second highest score answer alternative Very good, giving 85 score points, reached the highest rate of use, in more than half of the parents whereas Excellent is too high a score for most parents to give

their children. Still, one study showed a certain association with global behaviour of obstructive episodes in children with cleft palate or lip (98), and another separately scored domain of behaviour of ITQOL-97, the 12-item concept of General behaviour, has a better sensitivity.

Surprisingly, Change in health as a continuous recoded domain was negatively associated with length-of-stay by the first QoL survey. As shown by R. Oostenbrink et al., a chronic disease in other contexts seems to be associated with reported improved health compared with one year ago (83, 92). The negative association was only found for the fifth quintile of length-of-stay vs. the first quintile. However, although categorising a continuous variable into quintiles has disadvantages, a special non-linear association affecting the extreme long-stayers may be lost by analysis of the continuous variable of length-of-stay. If we study length-of-stay as a continuous variable depending on change-in-health as a categorical variable, the significance disappears, but Change in health as a dependent continuous variable is also negatively associated with length of stay as a continuous variable after adjustment for age and gender (results not shown). Thus, analysing Change in health as a continuous variable by robust regression as we presented in three articles seems relevant.

It is not clear to what extent the scoring differences are of clinical relevance, although we attempted to assess this by defining the minimal clinical relevant differences in paper xx. However, the fact that the changes generally point in the same direction, i.e. having undergone acute bronchiolitis have negative effects on QoL both nine months after acute disease and to the same degree at two years of age, and negative effects of four different aspects of disease severity in different domains of QoL, makes it very unlikely that the findings are random.

***Stress; represented by morning salivary cortisol***

The advantage of using a salivary marker is the feasibility and possibility to take samples in the patients' homes at due time, and the avoidance of triggering stress by the sampling itself, as shown by venipuncture in children with atopic dermatitis (75). We found that cortisol was raised in the acute stress situation of acute bronchiolitis, but that low cortisol was associated with low QoL, possibly related to chronic stress, in children after hospitalisation for acute bronchiolitis. Salivary  $\alpha$ -amylase could be an alternative marker of stress (99, 100), but probably not our first choice if we also want an assessment of the subjects' adrenal cortical function.

Morning salivary cortisol may be ambiguous as a stress marker and basal cortisol measurements have been considered inferior to dynamic testing (54). However, it has been shown that salivary morning cortisol in adult asthma patients receiving inhaled corticosteroids has potential as a screening method for detecting pronounced HPA- axis suppression (101).

By instructing the hospital staff and parents to take a sample as soon as possible after awakening, before the first meal, we achieved a simple procedure, more likely to be feasible in families with infants and two-year-old children making a larger participation and compliance more likely. If we had required an additional sample half an hour later, in order to more certainly catch a cortisol awakening response, the results could be likely to interfere with the family routines and need for time for meals, toilet visits, tooth brushing, time for siblings, need to hurry to the day-care centres etc. We collected single cortisol samples at each of the two ages, but we know there is notable intra-individual variability in salivary cortisol levels which is less apparent when collection is taken half an hour after awakening in subjects 8-65 years of age (102). Instead of waiting for a certain time after awakening, we found it more acceptable to take the samples as soon as possible rather than forcing infants and young children to wait eating for 30 minutes after awakening until their sample could be taken. Our

large sample is likely to compensate for noise by intra-individual variability. Furthermore, T. Nagakura et al. did not find any significant day-to-day variations between three morning samples taken at intervals of four to eight days (58).

### ***Asthma***

An advantage of applying rBO by parent reported episodes of bronchial obstruction as a proxy for asthma, is that we select children with symptoms varying over time. According to the GINA Pocket Guide for Health Professionals 2018, typical symptoms are wheeze, shortness of breath, chest tightness and cough, variable over time, but reversibility by beta-2-agonists may be absent in viral infections or during severe exacerbations. If we had required reversibility by beta-2-agonists in acute exacerbations as an objective criterion, we would be likely to exclude many children with asthma. Recurrent bronchial obstruction in children 0-2 years of age as a proxy for early asthma has been used in another study by V. Hovland et al. (103), and as mentioned, is in line with a Nordic consensus report on asthma from 2000 (10). Objective criteria would also have been more difficult to achieve in two-year-old children in a large scaled multi-centre study. Also, the sample size of our study can make objective criteria less important.

### ***Atopic dermatitis***

For the diagnosis of atopic dermatitis, we applied the Hanifin and Rajka criteria. Other diagnostic methods have later evolved, but in a study by D. De et al., Hanifin and Rajka's criteria were found superior to the UK working party diagnostic criteria, showing a higher sensitivity and positive predictive value (104).

### ***Allergic sensitisation***

Skin prick testing was the most widely used method of testing for allergy according to a systematic review on the definition of allergic diseases in children (105). It is well known that allergy cannot be diagnosed by testing alone, but must be confirmed by the history or provocation. In our study, a positive skin prick test was not necessarily confirmed. A reason

for finding little impact from skin prick tests on the associations between QoL and cortisol may be the low frequency of positive skin prick tests in our children.

***Asthma risk factors***

We analysed the impact of the asthma risk factors parental asthma, parental allergic rhinitis, atopic dermatitis and parental smoke at inclusion on QoL nine months later. Parental atopy and parental smoke has been shown to increase the risk of asthma in the offspring (106).

Atopic dermatitis in children doubles the risk of developing asthma, and one in three children with atopic dermatitis will develop asthma (107). Thus, the relationship we found between atopic dermatitis in infancy and QoL nine months later cannot be attributed only to development of asthma.

## CONCLUSIONS AND CLINICAL IMPLICATIONS

Were our hypotheses confirmed or rejected?

1. Are acute bronchiolitis and severity of the disease associated with QoL?

Acute bronchiolitis in infancy was associated with reduced QoL, particularly for the two domains Overall health and General health domains both nine months after the disease as well as at two years of age, as well as by reported improved health. The disease severity was associated with reduced QoL nine months later, especially in infants who had required ventilatory support or longer hospital stays. The clinical implications may be an increased alertness of parents and health staff to children who required ventilatory support by letting these children have a lower threshold for hospital admission or outpatient clinical care. On the other hand, a longer hospital stay was the only severity measure to indicate a lower Change in health compared to one year earlier nine months after inclusion. Therefore, letting the children stay hospitalised longer to make parents feel safer when objective criteria for moderate to severe disease are no longer present and the child drinks and sleeps well, may make parents more worried for their children's health for a longer period.

2. Is stress as measured by morning salivary cortisol associated with acute bronchiolitis and with development of asthma or other atopic diseases?

Acute bronchiolitis was associated with concurrent higher morning salivary cortisol, possibly due to acute stress induced by acute bronchiolitis, while at two-years of age rBO was associated with lower concurrent morning salivary cortisol levels. This suggests different mechanisms involved in acute and chronic respiratory disease, with a lower cortisol in children with asthma also in two year old children. We found no significant association between salivary cortisol and atopic dermatitis or allergic

sensitisation. Low morning cortisol in a two year old child with asthma-like symptoms may increase the suspicion that the child is developing asthma.

3. Is QoL associated with asthma or asthma development, risk factors for asthma and other atopic diseases in young children, with or without acute bronchiolitis in infancy?

QoL was reduced among children with rBO, as well inversely associated with increasing number of wheeze episodes and atopic dermatitis. A higher number of asthma risk factors at inclusion was associated with a lower QoL nine months later, including family cohesion. This may indicate a negative effect on QoL not only by asthma and atopic dermatitis, but from asthma risk factors overall, also before asthma has been diagnosed. Possibly, QoL measurement can be a helpful tool to identify children needing follow-up for a possible development of asthma or atopic dermatitis. In a child with at least three asthma risk factors, parents may possibly benefit from support and information about the stress this accumulation of risk factors may put on the family's cohesion.

4. Is there a link between QoL, morning salivary cortisol and atopic diseases that is influenced by moderate to severe acute bronchiolitis in infancy?

An association between low QoL and low morning salivary cortisol in the bronchiolitis group only was influenced by, but not fully explained by rBO. This indicates that factors other than rBO may influence the adrenal gland's capability of producing a morning cortisol peak after acute bronchiolitis in infancy. The clinical relevance of finding an association between low QoL and low morning salivary cortisol in children after acute bronchiolitis, but not in a control group with a higher frequency of parental allergic rhinitis and parental atopic dermatitis is uncertain, but



this is an exploratory study, confirming results of chronic stress in older children with asthma, with a negative association between stress and cortisol, as opposed to the high cortisol seen in non-atopic subjects exposed to stress. Our study shows that this process is detectable already in early childhood. It is our hope that this study can contribute to our understanding of the importance of a safe and child-friendly social environment in early life.

## Future perspectives:

Research of the influence of acute bronchiolitis in infancy on QoL through later childhood is required, including the significance of ventilation support and longer hospital stays. Further studies are also wanted for the effects of asthma on morning salivary cortisol and vice versa and the relationship with QoL later in childhood. Underlying mechanisms for associations between asthma and low cortisol should also be further clarified. It is also interesting to study the significance of more serious atopic dermatitis or three asthma risk factors on morning salivary cortisol and to what degree low QoL in early childhood can predict the degree of later asthma or other atopic disease. It is also desirable to examine what other factors than rBO after acute bronchiolitis that contributes to reduced QoL. The contribution of a safe and child-friendly social environment including reduction of parental stress and help for family cohesion on the risk of later atopic disease development should be elucidated. More should be done to find out which comes first, asthma or low morning salivary cortisol. If better measures to protect from RS-virus or other viral infections become real or more widely used, the effects on later QoL, morning salivary cortisol and atopic diseases should be studied.

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REGULAR ARTICLE

## Children hospitalised with bronchiolitis in the first year of life have a lower quality of life nine months later

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### Keywords

Acute bronchiolitis, Asthma, Atopic eczema, Quality of life, Risk factors

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### Received

13 March 2014; revised 20 June 2014;  
accepted 25 August 2014.

DOI:10.1111/apa.12792

The copyright line for this article was changed on 8 January 2016 after original online publication.

### ABSTRACT

**Aim:** Acute bronchiolitis increases the risk of asthma, and reduced quality of life (QoL) is reported in children with asthma and allergy. However, the impact of asthma risk factors on QoL is unclear. This study investigated whether bronchiolitis and common asthma risk factors in infancy had an influence on later QoL.

**Methods:** The parents of 209 infants recruited during hospitalisation for bronchiolitis at a mean age of 4 months, and 206 controls responded to the generic Infant Toddler Quality of Life Questionnaire 9 months later. We used robust regression analyses to assess the association between four asthma risk factors, atopic eczema, parental asthma, parental allergic rhinoconjunctivitis and second-hand smoke and QoL in the two groups.

**Results:** QoL was lower among children with previous bronchiolitis in the overall health and general health domains and lower in six of 13 domains in children with atopic eczema. Compared with no risk factors, children with previous bronchiolitis and three risk factors had lower scores in four domains, and control children with three risk factors had lower scores in three domains.

**Conclusion:** Having acute bronchiolitis, atopic eczema and three asthma risk factors were negatively associated with later QoL in early childhood.

### INTRODUCTION

Asthma is the most common chronic disease in childhood and is often preceded by acute bronchiolitis in infancy (1). Other common risk factors for asthma are atopic eczema (2), parental asthma (2), male gender (3) tobacco smoke exposure (4) and parental allergic rhinoconjunctivitis (5).

Health-related quality of life (QoL) reflects the severity of chronic diseases such as asthma (6,7) and is reduced in subjects with allergic rhinoconjunctivitis (8). Reduced QoL has also been reported in adolescents with asthma who are exposed to second-hand smoke (9) and adults exposed to maternal smoking during pregnancy (10). The general health Infant Toddler Quality of Life Questionnaire™ (ITQOL) was recently validated and applied to infants with obstructive airways disease (11–13) and other diseases (13). When the ITQOL was applied to 5000 infants in the Generation R study, QoL was reduced for the majority of domains and particularly for the general health, bodily pain and family activities domains in infants with asthma-like

symptoms (11). Similar reductions of QoL were reported 2–6 months after acute bronchiolitis (13). However, it is not known whether the presence of asthma risk factors *per se* induces a health burden, as reflected by reduced QoL in children younger than 24 months, or whether hospitalisation for acute bronchiolitis influences the susceptibility to reduced QoL.

The main aim of this study was to investigate whether bronchiolitis and common asthma risk factors were associated with later reductions in children's QoL and the

### Abbreviations

ITQOL, Infant Toddler Quality of Life Questionnaire™; ns, Not significant; QoL, Quality of life; SD, Standard deviation.

### Key notes

- This study investigated whether bronchiolitis and common asthma risk factors in infancy had an influence on later quality of life (QoL).
- The parents of 209 infants hospitalised for bronchiolitis at a mean age of 4 months, and 206 controls responded to the Infant Toddler Quality of Life Questionnaire 9 months later.
- Having acute bronchiolitis, atopic eczema and three asthma risk factors were negatively associated with later QoL in early childhood.

possible modification of this association by hospitalisation due to acute bronchiolitis in infancy. Secondly, we aimed to assess the relative impact of each of these risk factors as well as by the sum of risk factors on general health-related QoL in early childhood.

## METHODS AND SUBJECTS

### Study design

This study included 209 of the 404 children hospitalised during 2010–2011 for acute bronchiolitis at a mean age of 4.2 months (range 0–11 months) (14) in eight counties of the South-East Health Region of Norway. We also included 206 of the 240 control children recruited from a general population of infants of similar age in 2012. Inclusion criteria for the Bronchiolitis ALL-SE study (14) were as follows: clinical signs of bronchiolitis, as defined by Court (15), age below 12 months and a clinical score of four or more on a scale of zero to 10, where 10 was the worst. Exclusion criteria were any severe or chronic disease that might significantly influence the progression of acute bronchiolitis, more than one previous episode of obstructive airway disease or more than four weeks with lower airways disease symptoms and/or use of inhaled corticosteroids in the previous four weeks. The control population was recruited by randomly selecting infants aged up to 12 months from the national population registry who were living in two municipalities close to Oslo University Hospital and Østfold Hospital Trust, Fredrikstad. The exclusion criteria were any severe underlying disease, for example heart, lung, immunological, neurological or oncological disease. A medical history, including socio-economic factors and health-related issues in the subjects and their families, was obtained during enrolment.

The 97-question ITQOL™, version ITQOL-97 (ITQOL), was mailed to the parents of all 644 bronchiolitis patients and controls 8–9 months after enrolment and returned in stamped addressed envelopes.

The study was approved by the regional medical ethics committee and registered in the Norwegian bio bank registry. Informed written consent was obtained from parents of all the children. The clinical trial of the children with acute bronchiolitis (14) was registered with ClinicalTrials.gov number, NCT00817466. EudraCT number 2009-012667-34.

### Subjects

The 415 children (57.6% boys) in this study had a mean age of 14.0 months at QoL assessment, with a range of 8.4–23.3 months. These children were largely similar to the 229 whose parents did not return the QoL questionnaire, but were more likely to have Caucasian parents (fathers 92.0% vs. 86.7%,  $p = 0.001$ ) and a higher mean (SD) educational level [fathers 4.1 (0.9) vs. 3.8 (1.0),  $p < 0.001$ ]. The parents of the control children had a higher mean educational level, as well as higher rate of allergic rhinoconjunctivitis, than those of the hospitalised children. The control children were exposed to second-hand smoke significantly less often

than the hospitalised children, and their mean age at assessment was 0.7 months higher (Table 1). Eight children in the control group were reported to have had previous hospitalisation for acute bronchiolitis and were therefore reclassified as having undergone hospitalisation for acute bronchiolitis.

### Parental interview at enrolment

At enrolment, a paediatrician conducted a structured interview with the parents. Information was obtained about parental asthma and allergic rhinoconjunctivitis, previous respiratory symptoms, medications or atopic eczema in the child, indoor smoking in the home, ethnicity and socio-economic factors.

Second-hand smoke exposure was considered positive if the parents reported smoking in the home. Education was scored by the highest level in any of the parents into five categories, with category one denoting no schooling; two meaning primary school; three indicating secondary school; four showing higher education up to 3 years and five meaning higher education of more than 3 years.

### Quality of life questionnaire

The ITQOL (copyright holder HealthActCHQ Inc., Boston, MA, USA) includes 13 domains: overall health, physical abilities, growth and development, bodily pain/discomfort, temperament and moods, general behaviour, global behaviour, getting along, general health, change in health (compared with 1 year ago), parental impact – emotions,

**Table 1** Comparison of demographic and asthma risk factor data between bronchiolitis and control group responders

	Bronchiolitis N = 217	Controls N = 198	p-Value for difference
Males, n (%)	129 (59.4)	110 (55.6)	0.42
Parental asthma, n (%)	43 (24.2)	54 (27.3)	0.49
Parental allergic rhinoconjunctivitis, n (%)	<b>71 (35.3)</b>	<b>108 (54.5)</b>	<b>&lt;0.001</b>
Atopic eczema, n (%)	23 (11.1)	21 (10.6)	0.87
Second-hand smoke, n (%)	<b>32 (16.7)</b>	<b>6 (3.1)</b>	<b>&lt;0.001</b>
Caucasian mother, n (%)	190 (95.5)	189 (95.5)	0.99
Caucasian father, n (%)	190 (96.4)	184 (92.9)	0.12
Education of mother, mean (SD)	<b>4.1 (0.9)</b>	<b>4.6 (0.7)</b>	<b>&lt;0.001</b>
Education of father, mean (SD)	<b>3.9 (1.0)</b>	<b>4.3 (0.9)</b>	<b>&lt;0.001</b>
Age in months at inclusion, mean (SD)	<b>4.1 (2.8)</b>	<b>6.4 (3.4)</b>	<b>&lt;0.001</b>
Age in months at quality of life, mean (SD)	<b>13.7 (3.1)</b>	<b>14.4 (3.4)</b>	<b>0.02</b>

Significantly differing means with p-values in bold type.

parental impact – time and family cohesion. The domains were scored separately from 0 to 100 (best), except for change in health, which was scored from one to five (best). With permission from the copyright holder, we recalculated the change in health scores into a 0 to 100 (best) range. There was no overall summation score for the ITQOL. A validated Norwegian translation of the ITQOL (provided by the HealthActCHQ) was employed. One postal reminder was distributed after 2 weeks.

### Main outcomes, asthma risk factors and explanatory variables

The main outcomes were the 13 ITQOL domains. The four risk factors studied in the bronchiolitis and control groups separately were parental asthma, parental allergic rhinoconjunctivitis, second-hand smoke exposure and atopic eczema in the child. Gender and age at enrolment were entered as potential explanatory variables for QoL.

### Statistical analyses

Continuous data are presented by means and standard deviations with differences analysed by Student's *t*-test in case of normal distribution of data. The Welch test was applied for comparison of the means of non-normally distributed data like age at enrolment.

Categorical variables are given as numbers (n) and percentages, unless otherwise stated, and possible differences were assessed by Pearson's chi-square test. Due to non-normality of the distribution of the QoL scores and residuals, analyses of QoL data were made by Huber's M method of robust regression (16).

To estimate the relative impact of each risk factor on the ITQOL domains, we used Hosmer's manually backward elimination technique in multiple robust regression analysis, retaining age at inclusion and gender in all models (17). Ethnicity was not included in the multiple regression analysis, as the responding parents of the bronchiolitis and control groups did not differ significantly using this variable. All included variables were tested for confounding. Interactions and model assumptions were inspected using jackknife residuals and Akaike information criteria (AIC). The level of significance was set to  $p = 0.05$  for all analyses.

The 13 domains could not be regarded as independent of each other. Also, an interaction was found between bronchiolitis and some of the other risk factors for three of the domains (data not shown). Thus, the impact of risk factors on the QoL domains was analysed separately for the groups.

Adjustment of *p*-values for multiple variables was not performed for reasons explained in the discussion. The SPSS PASW Statistics 18 (IBM Corporation, Armonk, NY, USA) and the Number Cruncher Statistical System (NCSS, Kaysville, UT, USA) version 2007 were used in the analyses.

## RESULTS

Characteristics of the 217 children with previous hospitalisation for acute bronchiolitis and the 198 controls are

given in Table 1. The QoL was significantly associated with three domains in children with previous bronchiolitis (Table 2), compared with those without bronchiolitis. The mean (95% confidence interval) differences (regression coefficients) in QoL scores were most pronounced for general health at  $-11.6$  ( $-14.0$  to  $-9.2$ ), followed by overall health at  $-5.8$  ( $-8.3$  to  $-3.2$ ), whereas a significantly higher score was observed for change in health at  $4.6$  ( $1.0$ – $8.3$ ) among children with previous bronchiolitis. The other domains were not significantly associated with bronchiolitis.

Atopic eczema had the most widespread negative impact on QoL after adjusting for age and gender, affecting five domains in the bronchiolitis group (Fig. 1) and six domains in the control group (Fig. S1). In both groups, the QoL domains, overall health, physical abilities and temperament and moods, were negatively affected by atopic eczema. Parental asthma negatively affected one domain in the control group. Exposure to second-hand smoke was negatively associated with one domain in the controls and two domains in those with previous bronchiolitis. The most pronounced impact was found in the domain of change in health among children with previous bronchiolitis, with higher scores in those with atopic eczema and lower scores in those with second-hand smoke exposure.

Girls with previous bronchiolitis had significantly higher scores than boys in the domain bodily pain/discomfort. Age at inclusion was negatively associated with general behaviour in the control group.

The number of common asthma risk factors did not differ significantly in the bronchiolitis and the control children, respectively, with no risk factor in 41.3% versus 33.3%, one risk factor in 38.9% versus 40.4%, two risk factors in 16.8% versus 23.7% and three risk factors in 2.9% versus 2.5%. Overall, the most common risk factor (n, %) was parental

**Table 2** Weighted means\* of quality of life (QoL) scores of the two groups (95% confidence intervals); *p*-values referring to differences between the groups

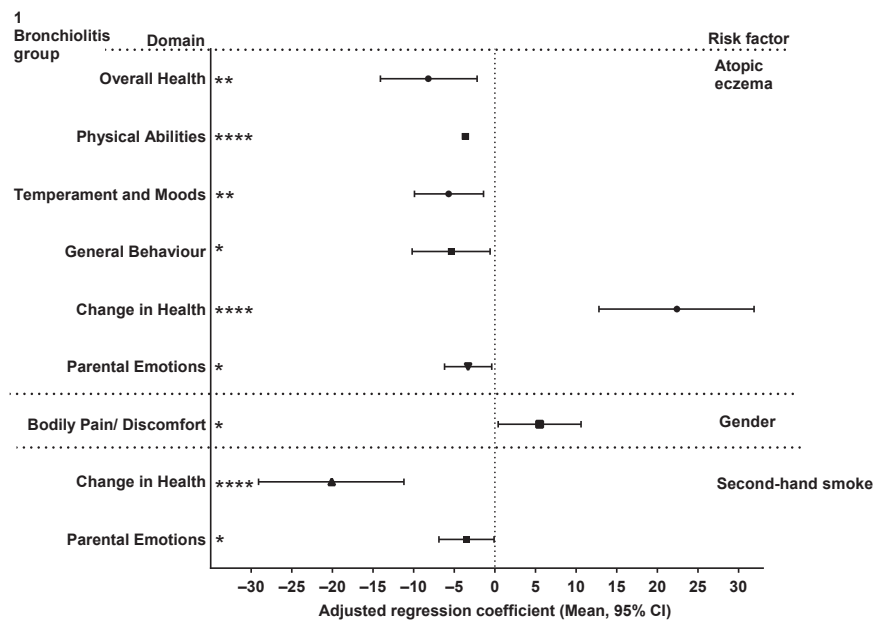
QoL domain	Bronchiolitis	Controls
Overall health	<b>85.4 (83.6, 87.2)</b>	<b>91.2 (89.4, 93.0)</b>
Physical abilities	99.5 (99.8, 100.0)	100.0 (99.9, 100.1)
Growth and development	93.9 (92.8, 94.9)	93.7 (92.7, 94.8)
Bodily pain/discomfort	72.9 (70.7, 75.1)	74.0 (71.8, 76.3)
Temperament and moods	83.1 (82.0, 84.3)	83.1 (81.9, 84.3)
General behaviour <sup>†</sup>	88.0 (86.4, 89.5)	89.3 (87.7, 90.9)
Overall behaviour <sup>†</sup>	88.8 (86.6, 91.0)	88.4 (86.1, 90.6)
Getting along <sup>†</sup>	80.0 (78.6, 81.3)	78.4 (77.0, 79.7)
General health	<b>70.2 (68.5, 71.9)</b>	<b>81.8 (80.0, 83.5)</b>
Change in health <sup>†‡</sup>	<b>56.0 (48.9, 53.8)</b>	<b>51.4 (48.9, 53.8)</b>
Parental emotions	93.6 (92.7, 94.5)	93.5 (92.6, 94.4)
Parental time	94.1 (92.9, 95.2)	94.1 (93.0, 95.3)
Family cohesion	85.0 (85.0, 85.0)	85.0 (85.0, 85.0)

\*Means weighted by robust regression by Huber's M method.

<sup>†</sup>Only for children older than 12 months of age.

<sup>‡</sup>Recorded from scores 1–5 to 0–100.

Significantly different values in bold type.



**Figure 1** Significant associations between quality of life and asthma risk factors are shown in children who were previously hospitalised with acute bronchiolitis. The association with each significant risk factor is given as the regression coefficient (95% confidence interval), adjusted for age and gender in multiple robust regression analyses. Risk factors not shown were excluded by step-down procedure. \*0.05 > p ≥ 0.01, \*\*0.01 > p ≥ 0.001, \*\*\*0.001 > p ≥ 0.0001, \*\*\*\*p < 0.0001.

allergic rhinoconjunctivitis (179, 43.1%), followed by parental asthma (97, 23.4%), diagnosed atopic eczema in the child (44, 10.6%) and second-hand smoke (38, 9.2%). No subjects had all four risk factors.

Compared with no risk factors, the presence of increasing numbers of asthma risk factors was associated with lower QoL in both groups, as shown in Table S1. In children with previous bronchiolitis, QoL in one and four domains was significantly reduced with the presence of two or three risk factors, respectively. Among the controls, the presence of one risk factor was associated with lower scores in one domain, whereas two or three risk factors were associated with reduced scores in three domains.

The largest reduction in score was seen for family cohesion in children with previous bronchiolitis and three asthma risk factors, followed by bodily pain/discomfort and general health in those with three risk factors in the control group (Table S1).

## DISCUSSION

Hospitalisation for acute bronchiolitis, particularly in children who also had atopic eczema, was associated with reduced infant QoL 9 months later. A similar effect was seen by an increasing number of asthma risk factors.

Our finding that QoL was reduced 9 months after hospitalisation for acute bronchiolitis is in line with a previous Dutch study during early childhood by Spuijbroek et al. (13) and Backman et al. (1) in adulthood. In common with the present study, the Dutch study also reported significant reduction in general health 2–6 months after respiratory syncytial virus bronchiolitis in 47 children (13). Wheezing

and asthma-like symptoms have previously been associated with reduced QoL (11–13,18) although recurrent wheeze is likely to represent a different entity of obstructive airways disease than the presently reported acute bronchiolitis.

Our study is the first to assess the role of asthma risk factors on generic QoL in early childhood. Atopic eczema at the time of enrolment, included as one of the asthma risk factors, was associated with reduced general health-related QoL in line with findings in older children (19,20) using disease-specific QoL questionnaires (21). In children aged three to 84 months, using the Infants' Dermatitis Quality of Life Index, a negative correlation was found between the presence and degree of atopic eczema and mood and sleep (20). However, this index was only designed and validated for children with atopic eczema. Accordingly, many questions were inappropriate for healthy children. Disease-specific and general health QoL instruments are not directly comparable, possibly explaining some of the discrepancy in magnitude of associations. The presently observed negative impact on QoL by atopic eczema of up to 11 percentage points or score points is less than the 33% reduction in QoL observed in 5 to 16-year-old Scottish children with generalised atopic eczema, but more in line with the 19% reduction in QoL with localised eczema (22). In comparison, Beattie et al. (22) reported a mean reduction of QoL for asthma of 28%. The apparent discrepancy between the Scottish study and our study may be due to our use of the presence or not of atopic eczema recorded at enrolment as a predictor for later QoL, without assessing eczema at the time of completing the QoL questionnaire. We did not distinguish between generalised and localised eczema.



The other three asthma risk factors – parental asthma, parental allergic rhinoconjunctivitis and second-hand smoke exposure – were to a lesser extent independently associated with QoL.

The present study showed that increasing numbers of asthma risk factors were associated with lower general health-related outcomes. As shown, parental allergic rhinoconjunctivitis was not independently associated with any of the QoL domains, and parental asthma was only associated with one domain. Still, having many risk factors together with rhinoconjunctivitis may reflect a greater untoward health load that influences how well the parents cope with, and perceive, their child's health as well as their own QoL. Our data suggest that hospitalisation for acute bronchiolitis may modify the association between QoL and common asthma risk factors. Atopic eczema was negatively associated with QoL in the control group, whereas second-hand smoke exposure showed a negative association in the bronchiolitis group. We speculate that having experienced a severe lower respiratory disease leading to hospitalisation during infancy may override a potential limited effect by a common asthma risk factor, as suggested by the impact shown in Figure 1. A large general cohort study including sufficient number of children hospitalised for acute bronchiolitis would be necessary to confirm or contradict our findings.

The present study is strengthened by the prospective assessment of asthma risk factors at enrolment for the two groups of children, thus eliminating the risk of recall bias at the time of reporting QoL 9 months later. The inclusion of a randomly selected control group from a general population of similar age enabled us to study the potential modifying effect of acute bronchiolitis exerted by asthma risk factors on later QoL. To ensure that all children who had experienced acute bronchiolitis were classified correctly in stratified analyses, the eight control subjects who had been hospitalised for bronchiolitis were classified together with the bronchiolitis group. This provided a more stringent opportunity to assess the role of bronchiolitis in later QoL than if they had been classified with the controls. The ITQOL is carefully validated in general infants and child populations (12) as well as for young children with diseases (11,13) relevant for the present study.

The study has limitations that may influence the interpretation of our results. The response rate in the bronchiolitis group was 52%, which appeared to skew the respondents towards native families with higher education than the nonrespondents. The respondents from the bronchiolitis group differed less from the control group than the nonrespondents from the bronchiolitis group did with respect to parents' education and did not differ significantly from the control group by ethnicity. Our ability to generalise the findings to a truly random population may be restricted. The interpretation of the role of risk factors in reduced QoL may be limited within populations of less highly educated parents or non-Caucasian families.

Adjusting for multiple testing is debated. However, the use of Bonferroni adjustment in multiple regression

analyses is designed for outcome variables that are independent of each other, which is not the case with each of the ITQOL domains being measured in the same individual. The negative influence of atopic eczema on ITQOL was found in a high number of domains, thus strengthening the likelihood of statistically as well as clinically significant associations without adjusting for multiple testing.

## CONCLUSION

Having acute hospitalised bronchiolitis, atopic dermatitis and three common asthma risk factors during infancy were associated with lower general health-related QoL in early childhood.

## ACKNOWLEDGEMENTS

We warmly acknowledge all participants in the Bronchiolitis Study Group, as well as the several hundred study staff that were involved in recruiting patients and running the study. These include, but are not limited to, Sabine Kristin Brüggemann-Pieper, Vestre Viken Hospital Trust, Ane Charlotte Brun, Vestfold Hospital Trust, Hanne Engen, Telemark Hospital Trust, Leif Eskedal, Sørlandet Hospital Trust, Marius Haavaldsen, Østfold Hospital Trust, Christian Siva, Vestfold Hospital Trust, Truls Vikin, Innlandet Hospital Trust, Solveig Knutsen and Live S. Nordhagen, Oslo University Hospital HF. We thank Sigmund Rolfjord for help with setting up syntaxes in SPSS for ITQOL scoring. The study was performed within ORAACLE (the Oslo Research Group of Asthma and Allergy in Childhood; the Lung and Environment), a member of MeDALL (Mechanisms of the Development of ALLergy), a collaborative project conducted with support from the European Union under the Health Cooperation Work Programme of the 7th Framework programme (grant agreement No. 261357).

## CONFLICTS OF INTERESTS AND FUNDING

None of the authors have reported any conflict of interest related to the present study. The bronchiolitis trial was run in eight paediatric departments without commercial funding. The first author has a 50% fellowship from the Innlandet Hospital Trust's Research Fund.

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#### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1** The impact on QoL domains by increasing number of the risk factors atopic eczema, parental asthma, parental allergic rhinoconjunctivitis and second-hand smoke as the effect on the change of domain score compared to having no risk factor (0), mean (95% confidence intervals) for children admitted to hospital for acute bronchiolitis (B) and control children (C).

**Figure S1** Significant associations between QoL and asthma risk factors are shown in children from the general population sample who were not previously hospitalised with acute bronchiolitis.



Supporting Information online

**Table S 1** The impact on QoL domains by increasing number of the risk factors atopic eczema, parental asthma, parental allergic rhinoconjunctivitis and second-hand smoke as the effect on the change of domain score compared to having no risk factor (0), mean (95% confidence intervals) for children admitted to hospital for acute bronchiolitis (B) and control children (C). No child had all four risk factor present.

Domain	Group <sup>1</sup>	No risk factor	Change score 1 risk factor	Change score 2 risk factors	Change score 3 risk factors
Overall	B	85.7 (82.8, 88.7)	1.1 (-3.2, 5.5)	-1.4 (-6.9, 4.1)	-9.1 (-20.1, 2.0)
Health	C	91.9 (89.0, 94.9)	-0.3 (-4.2, 3.6)	-3.8 (-8.4, 0.8)	-9.4 (-20.8, 2.1)
Physical Abilities	B	99.6 ((99.3, 100.0)	0.1 (-0.5, 0.7)	-0.6 (-1.4 0.3)	<b>-7.1 (-9.1, -5.1)</b>
	C	100.0 (100.0, 100.0)	0.0 (0.0, 0.0)	<b>-0.3 (-0.7, -0.0)</b>	-0.1 (-0.6, 0.4)
Growth and Development	B	94.3 (92.6, 95.9)	-0.8 (-3.1, 1.6)	0.8 (-2.3, 3.8)	-1.5 (-7.7, 4.8)
	C	96.1 (94.4, 97.8)	<b>-2.5 (-4.8, -0.2)</b>	<b>-4.2 (-6.9, -1.5)</b>	-4.4 (-11.1, 2.2)
Bodily Pain/ Discomfort	B	73.5 (69.8, 77.2)	1.4 (-4.0, 6.7)	-5.9 (-12.9, 1.1)	-4.0 (-18.5, 10.4)
	C	76.8 (73.3, 80.3)	-4.7 (-9.5, 0.0)	-2.3 (-7.8, 3.1)	<b>-18.5 (-32.9, -4.0)</b>
Temperament and Moods	B	83.2 (81.2, 85.2)	1.8 (-1.1, 4.7)	<b>-5.5 (-9.2, -1.7)</b>	-2.7 (-10.4, 5.0)
	C	83.6 (81.9, 85.4)	0.0 (-2.3, 2.4)	-1.4 (-4.1, 1.4)	<b>-10.1 (-17.0, -3.2)</b>
General Behaviour	B	87.5 (84.8, 90.2)	2.0 (-1.9, 5.9)	-1.4 (-6.2, 3.4)	0.0 (-9.9, 9.9)
	C	87.7 (85.0, 90.3)	3.2 (-0.2, 6.6)	0.5 (-3.5, 4.6)	1.9 (-6.8, 10.6)
Overall Behaviour	B	88.0 (85.8, 93.2)	2.0 (-2.7, 6.6)	-1.3 (-7.1, 4.4)	-6.5 (-18.6, 5.6)
	C	88.5 (84.6, 92.4)	2.6 (-2.5, 7.6)	-1.5 (-7.6, 4.5)	4.0 (-8.8, 16.9)
Getting	B	79.6 (77.3, 81.9)	1.4 (-1.9, 4.7)	-1.1 (-5.3, 3.0)	-0.9 (-9.3, 7.6)

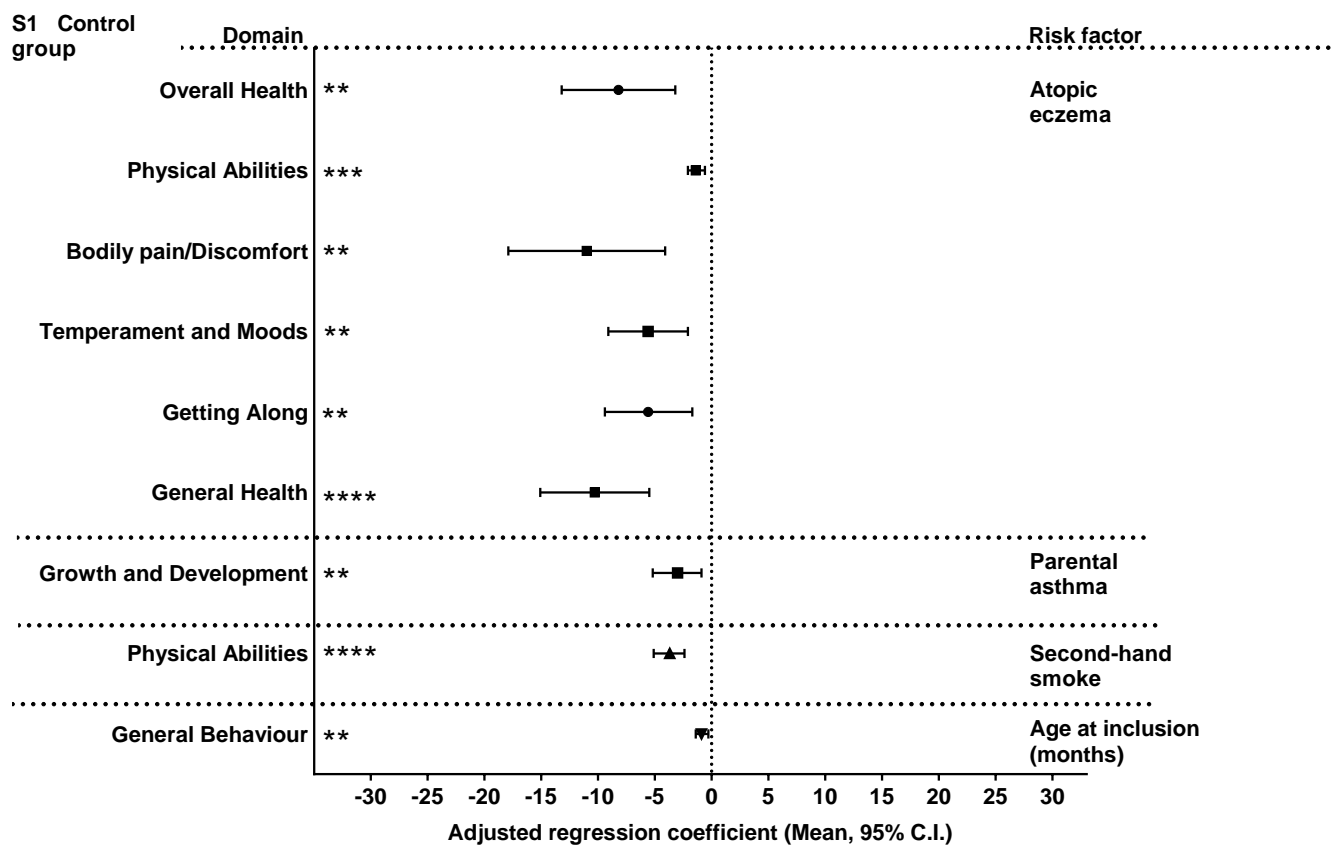
Along	C	77.4 (75.1, 79.8)	1.6 (-1.4, 4.7)	1.1 (-2.5, 4.7)	-1.3 (-9.3, 6.6)
General	B	71.1 (68.0, 74.1)	-0.6 (-5.0, 3.7)	-4.7 (-10.4, 1.0)	-2.6 (-14.4, 9.2)
Health	C	84.2 (81.7, 86.7)	-1.8 (-5.2, 1.7)	<b>-5.1 (-9.1, -1.1)</b>	<b>-11.5 (-21.0, -1.9)</b>
Change in	B	65.2 (57.9, 72.5)	-2.4 (-12.9, 8.1)	-5.0 (-18.2, 8.3)	-15.2 (-42.8,
Health					12.5)
	C	50.0 (50.0, 50.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Parental	B	93.7 (92.2, 95.1)	1.0 (-1.0, 3.1)	-2.5 (-5.2, 0.2)	<b>-8.0 (-14.4, -1.5)</b>
Emotions	C	93.9 (92.3, 95.5)	-0.6 (-2.7, 1.6)	-2.1 (-4.6, 0.5)	-5.4 (-11.4, 0.7)
Parental	B	94.3 (92.4, 96.2)	0.1 (-2.6, 2.9)	-1.9 (-5.5, 1.6)	<b>-10.2, -17.8, -</b>
Time					<b>2.5)</b>
	C	95.0 (93.1, 96.9)	-1.2 (-3.7, 1.3)	-1.1 (-4.0, 1.8)	-4.5 (-11.6, 2.6)
Family	B	85.0 (82.5, 87.5)	0.7 (-2.9, 4.4)	5.3 (0.9, 9.8)	<b>-21.7 (-32.5, -</b>
Cohesion					<b>10.9)</b>
	C	85.0 (85.0, 85.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)

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### Legend for figure S1

Significant associations between QoL and asthma risk factors are shown in children from the general population sample who were not previously hospitalised with acute bronchiolitis.

Methods and adjustments are made as for the bronchiolitis children, see legend for figure 1.









REGULAR ARTICLE

# The severity of acute bronchiolitis in infants was associated with quality of life nine months later

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## Keywords

Acute bronchiolitis, Asthma, Infants, Quality of life, Severity

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## Received

8 July 2015; revised 2 February 2016; accepted 7 March 2016.

DOI:10.1111/apa.13396

The study was performed within ORACLE (the Oslo Research Group of Asthma and Allergy in Childhood; the Lung and Environment).

## INTRODUCTION

Acute infant bronchiolitis is associated with later wheeze and asthma in children (1–3) and adults (1,4). Bronchodilator treatment of acute bronchiolitis has been found to be ineffective, whereas supportive care such as oxygen supplementation, nasogastric feeding and ventilatory support may be required (5,6). The severity of bronchiolitis has been assessed by inpatient status, length of hospital stay (LOS) (7,8) and by the use of general and ventilatory supportive treatment (9–11). Severity characteristics at the time of hospital admission include the presence of inspiratory retractions, wheezing, respiratory rate and oxygen saturation (12,13), which may predict the length of hospital stay (11).

Health-related quality of life (QoL) measures may quantify the individuals' capability to adapt to illness across social, emotional and physical functioning as well as how much their illness interferes with daily life (14). Hence, measuring QoL is an important supplement to clinical disease assessment.

Infant bronchiolitis has been associated with later reduced QoL in children (15–17) and adults (4). Bont et al. (17) reported that QoL at three years of age was associated with the severity of postbronchiolitis wheezing, but not with age, gender, gestational age or mechanical ventilation

## ABSTRACT

**Aim:** Acute bronchiolitis in infancy increases the risk of later asthma and reduced health-related quality of life (QoL). We aimed to see whether the severity of acute bronchiolitis in the first year of life was associated with QoL nine months later.

**Methods:** The parents of 209 of 404 of children hospitalised for acute bronchiolitis in eight paediatric departments in south-east Norway at a mean four months of age (range 0–12 months) completed the Infant/Toddler Quality of Life Questionnaire sent by mail nine months after the acute illness. Disease severity was measured by length of stay and the need for supportive treatment. Interactions with gender, inclusion age, prematurity, maternal ethnicity and maternal education were examined.

**Results:** Reduced QoL in four domains was associated with increased length of stay and need for ventilatory support. Physical abilities and general health were associated with both severity markers, whereas bodily pain and discomfort and change in health were associated with length of stay. Ventilatory support was more negatively associated with QoL than atopic eczema and also associated with reduced parental emotions and parental time.

**Conclusion:** The severity of acute bronchiolitis in infants was associated with reduced QoL nine months later.

during acute bronchiolitis. Wheezing illness (18) and asthma-like symptoms (19) have been associated with reduced QoL in infants and young children.

Using the 97 question version of the Infant/Toddler Quality of Life Questionnaire (ITQOL-97) (20), we have shown that hospitalisation for acute bronchiolitis as well as asthma risk factors, including atopic eczema in infancy, were associated with reduced general QoL nine months later (21). Apart from Bont's study (17), we were not aware of any studies that had investigated whether the severity of acute bronchiolitis was associated with later QoL. Disease

## Key notes

- We explored whether the severity of acute bronchiolitis in the first year of life was later associated with reduced health-related quality of life (QoL).
- The parents of 209 infants completed the Infant/Toddler Quality of Life Questionnaire nine months after they were hospitalised.
- Increasing severity was associated with lower QoL in the domains physical abilities, general health, bodily pain and discomfort, change in health, parental emotions and parental time.

severity during infancy may affect the parents' later perception of health and disease in young children. This has been shown for wheezing illness (18). Therefore, we aimed to investigate whether the severity of acute bronchiolitis in infants was associated with reduced QoL nine months later, primarily by assessing the severity of acute bronchiolitis by LOS and the need for supportive treatment and secondarily by the severity of bronchiolitis assessed upon admission to hospital.

## METHODS

### Study design

A multicentre, randomised clinical trial, registered as the Bronchiolitis All-Study, south-east Norway (9), compared the effect of inhaled racemic adrenaline versus saline and on demand versus fixed schedule inhalation strategies in infants from January 2010 through May 2011. The infants were recruited from eight paediatric hospital departments in the south-east health region of Norway. The inclusion criteria were that they needed to be less than 12 months of age and have clinical signs of moderate-to-severe bronchiolitis, with a clinical score of at least four on a scale from 0 to 10, with 10 being the worst (Table 1) (9,22). The exclusion criteria were severe underlying disease, more than one episode of previous wheeze, more than four weeks of continuous lower airway symptoms, such as a cough, and the use of corticosteroids in the previous four weeks.

At inclusion, the parents underwent structured paediatrician-guided interviews, including information on the patient and family medical history and sociodemographic factors. Oxygen saturation (SpO<sub>2</sub>) was measured transcutaneously with a pulse oximeter. The use of supportive treatment, defined as oxygen supplementation, nasogastric feeding tube or ventilatory support, was recorded daily and verified from patient record reviews. The ITQOL questionnaires were sent by mail to the parents of the bronchiolitis children about nine months after hospitalisation (mean 9.6 months, 95% confidence interval (95% CI) 9.4–9.8 months). Written informed consent was obtained from at least one parent of each child prior to inclusion. If the questionnaires were not returned within two weeks, another questionnaire was sent out once.

The study was approved by the Regional Committee for Medical and Health Research Ethics and by the Norwegian

Medicines Agency and was registered in the Norwegian Biobank Registry. The study was audited by the Norwegian Medicines Agency in 2011. The trial was registered in ClinicalTrials.gov (NCT00817466) and EudraCT (2009-012667-34).

### Subjects

This study included the 209 children whose parents returned the ITQOL, who represented 52% of the 404 included in the randomised clinical trial. The children (60% boys) had a median age of 3.3 months on hospital admission (Table 2), and a median (range) age of 13.0 (8.4–23.3) months at the time of the QoL assessment. The median (range) LOS was 67.4 (2.0–398.1) hours. All 14 children requiring ventilatory support received noninvasive ventilation by continuous positive airways pressure (CPAP), and none received ventilator treatment.

With the exception of more Caucasian mothers and higher parental education among the questionnaire respondents, the baseline characteristics were comparable in the respondents and nonrespondents (Table 2).

### Health-related quality of life

The ITQOL-97 consists of 97 questions divided into 13 domains: overall health, physical abilities, growth and development, bodily pain/discomfort, temperament and moods, general behaviour, overall behaviour, getting along, general health, change in health, parental impact (emotions), parental impact (time) and family cohesion. The first ten domains are related to the children, whereas the domains parental impact (emotions) and parental impact (time) are based on questions about the parents' worries and time limitations attributed to their children's health. The three behaviour domains, namely general behaviour, overall behaviour and getting along, and the change in health domain, are only suitable for children older than 12 months of age. The domains are scored from 0 to 100 (best), with no overall score. For the domain change in health, which reflects the parent's perception of their children's health compared to one year ago, the score from 1 to 5 (best) was recoded into 0–100 by permission of the copyright holder (HealthActCHQ Inc, Massachusetts, USA). A score of 50 for change in health indicates unchanged health from one year earlier. A validated translation into Norwegian, provided by the copyright holder, was applied.

**Table 1** The clinical score, based on Skjerven et al. (9) and Kristjansson et al. (22)

	Score 0	Score 1	Score 2
Respiratory rate (breaths/min)	<40	40–60	>60
Respiratory chest recessions	None	Moderate costodiaphragmatic	Severe As 1, +rib and jugular retraction
Auscultatory breath sounds	Vesicular	Wheeze + rales/rhonchi	Faint ± severe wheeze ± pronounced rales and rhonchi
Skin colour	Normal	Pallor	Cyanosis
General condition	Not affected	Moderately affected	Severely affected



**Table 2** Baseline characteristics of questionnaire respondents versus nonrespondents

	Respondents n = 209	Nonrespondents n = 195	p Value
Boys, n (%)	125 (59.8%)	115 (59.0%)	0.87
Age hospitalised (months), median (range)	3.3 (0.2–11.9)	3.9 (0.3–12.0)	0.47
Moderate or severe bronchiolitis, n (%)	105 (50.2%)	100 (51.3%)	0.83
Length of stay, hours, median (range)	67.4 (2.0–398.1)	64.2 (0.5–408.1)	0.63
Clinical score at inclusion, median (range) <sup>†</sup>	5.0 (4–9)	5.0 (4–9)	0.999
Oxygen saturation <92% at inclusion, n (%)	16 (8.0%)	14 (7.4%)	0.85
Mother Caucasian, n (%)	<b>198 (94.7%)</b>	<b>170 (87.2%)</b>	<b>0.008</b>
Father Caucasian, n (%)	181 (86.6%)	158 (81.0%)	0.13
Maternal education <sup>‡</sup> , median (range)	<b>4.0 (1–5)</b>	<b>3.0 (1–5)</b>	<b>&lt;0.001</b>
Paternal education <sup>‡</sup> , median (range)	<b>4.0 (1–5)</b>	<b>4.0 (2–5)</b>	<b>0.028</b>
Birthweight, kg, mean (SD)	3.5 (0.6)	3.4 (0.7)	0.057
Prematurity <sup>§</sup> , n (%)	19 (9.1%)	20 (10.3%)	0.69

Characteristics with significant differences in bold.

<sup>†</sup>Clinical score at inclusion – see definition in Table 2.

<sup>‡</sup>The parents' education level was scored by the following categories: 1 = no schooling; 2 = primary school; 3 = secondary school; 4 = higher education up to three years; 5 = higher education more than three years.

<sup>§</sup>Prematurity: Gestational age at birth <37 weeks.

### Severity of acute bronchiolitis

The severity of bronchiolitis was assessed by four criteria: LOS, bronchiolitis severity by need for supportive treatment, SpO<sub>2</sub> and clinical score upon inclusion in the study.

LOS was defined as the time from the first study inhalation until discharge from the hospital, as recorded in the medical record for each patient, and given by quintiles, with the lowest representing the shortest LOS.

Bronchiolitis severity was categorised into three discrete groups:

1. No supportive treatment: no nasogastric tube feeding, extra oxygen supply or ventilatory support given,
2. Supportive treatment: supportive treatment, but no ventilatory support given,
3. Ventilatory support: ventilatory support given, regardless of other supportive treatment.

The SpO<sub>2</sub> at study inclusion was reported quantitatively.

The clinical score at study inclusion (Table S1) was reported quantitatively from 4 to 10, as the inclusion criteria required a score of at least four.

### Outcomes

The main outcomes were the 13 domains of QoL reported from 1 to 100, with the highest values indicating a better QoL.

### Statistical analyses

Groups were compared by Pearson's chi-square tests for categorical data and nonparametric tests for numerical variables unless otherwise stated.

All analyses with QoL as outcomes were analysed by linear robust regression by Huber's M-method (23), due to non-normality of the results and residuals, as well as the logarithms of the results. Data are presented as weighted

means with 95% confidence intervals (95% CI). We calculated the percentage point reductions in scores for the domains that were significantly associated with the severity variables to estimate the relative association with QoL. Possible interactions between the severity measures, gender, age at inclusion, maternal education and ethnicity as well as prematurity were assessed by multiple robust regression, including Hosmer's step-down procedure (24), a priori retaining age and gender in the analyses, and by two-way analysis including variance analysis. After the step-down procedure, we finally selected variables with p values of less than 0.3. Confounding was considered significant if including the variable led to a minimum of a 25% change in the result (24).

Because of non-normality of LOS, quintiles were used in the analysis. The high number of domains, 13, in the QoL questionnaire leads to multiple analyses. The ITQoL domains are not independent from each other and that is why we chose not to adjust the p values for multiple analyses.

The level of statistical significance was set to 0.05 (5%) for all analyses. Analyses were performed with the IBM SPSS Statistics 20 (IBM Corporation, New York, USA) and the version 2007 of the Number Cruncher Statistical System (NCSS Kaysville, Utah, USA).

### RESULTS

The LOS varied from 2 to 398 hours, with quintiles of all children ranging from 0.5 to 23.0 hours (lowest) and 124.9 to 408. One hour (highest). No supportive treatment was given to 104 infants, while 91 received oxygen or feeding support and 14 also received ventilatory support. The youngest children were more likely to have more severe disease, with 27 of the 38 children (71.1%) within the highest quintile of LOS and 11 of the 14 children needing

ventilatory support (78.6%) being younger than three months of age. Although prematurity was negatively associated with six domains in bivariate analyses, prematurity did not significantly confound the associations between bronchiolitis severity and QoL (data not shown). Age at hospitalisation was not associated with QoL in bivariate analyses.

**LOS and need for supportive treatment**

Children with the longest LOS (5th quintile) had significantly reduced QoL in four domains, with a further tendency for reduced QoL in the overall health domain, significant for the 3rd quintile only (Table 3, Fig. 1). The reductions in QoL observed with increasing LOS (Fig. 1) were also significant after adjustment for age at hospitalisation and gender (Table 3) and were significant for the three highest quintiles in relation to the general health and

for two quintiles in the bodily pain and discomfort domains, respectively. The tendency for less improvement in health with increasing LOS compared to the lowest quintile was only significant in the highest quintile.

Children who received ventilatory support had significantly reduced QoL in four domains, while receiving supportive treatment was associated with reduced general health nine months later (Fig. 2), also after adjusting for age at inclusion and gender (Table 4).

The potential modifying effect of having experienced one episode of bronchial obstruction on the associations between acute bronchiolitis and later QoL was analysed by robust regression with and without adjustment for age at inclusion and gender. No significant effect was found for any of the associations, and the results for LOS and the need for supportive treatment are shown in Tables 3 and 4.

**Table 3** Significant associations between LOS and QoL domains<sup>†‡§¶</sup>, adjusted

QoL domain		1st quintile (0.5–23.0 hours) N = 38	2nd quintile (23.0–47.9 hours) N = 39	3rd quintile (47.9–75.6 hours) N = 51	4th quintile (75.6–124.9 hours) N = 43	5th quintile (124.9–408.1 hours) N = 38
Overall health	Adjusted for age and gender only	88.9 (83.9, 94.0)	−4.6 (−11.3, 2.2)	−6.4 (−12.6, −0.1)*	−3.6 (−10.0, 2.8)	−5.6 (−12.5, 1.3)
	Adjusted for one previous obstruction <sup>§</sup>	92.0 (85.7, 98.3)	−6.0 (−12.6, 0.5)	−4.1 (−10.2, 1.9)	−4.0 (−10.2, 2.2)	−4.5 (−11.2, 2.2)
Physical abilities <sup>¶¶</sup>	Adjusted for age and gender only	99.8 (99.5, 100.3)	0.1 (−1.2, 1.5)	−0.5 (−1.8, 0.8)	−0.6 (−2.0, 0.7)	−2.6 (−4.2, −1.0)**
	Adjusted for one previous obstruction	98.6 (97.1, 100.1)	0.2 (−1.3, 1.8)	−0.4 (−1.9, 1.0)	−0.6 (−2.1, 0.9)	−1.8 (−3.4, −0.1)*
Bodily pain/discomfort	Adjusted for age and gender only	79.9 (74.5, 85.2)	−4.0 (−12.5, 4.5)	−8.3 (−16.2, −0.4)*	−6.3 (−14.5, 1.9)	−9.5 (−18.2, −0.8)*
	Adjusted for one previous obstruction	74.6 (65.8, 83.4)	−2.7 (−11.9, 6.4)	−7.0 (−15.3, 1.3)	−4.3 (−12.9, 4.4)	−5.0 (−14.3, 4.2)
General health	Adjusted for age and gender only	75.3 (70.5, 80.1)	−5.0 (−11.7, 1.8)	−6.6 (−12.8, −0.3)*	−7.0 (−13.6, −0.4)*	−9.9 (−16.9, −3.0)**
	Adjusted for one previous obstruction	76.0 (69.0, 83.0)	−4.8 (−12.1, 2.4)	−5.5 (−12.1, 1.2)	−7.4 (−14.4, −0.4)*	−8.6 (−16.1, −1.1)*
Change in health	Adjusted for age and gender only	73.1 (63.0, 83.1)	−12.1 (−26.1, 1.9)	−12.5 (−25.9, 0.9)	−8.6 (−22.3, 5.2)	−25.7 (−43.3, −8.2)**
	Adjusted for one previous obstruction	81.1 (64.4, 97.8)	−16.5 (−31.7, −1.3)*	−14.5 (−28.6, −0.5)*	−13.4 (−28.4, 0.6)	−27.8 (−46.4, −9.1)**

\*0.01 ≤ p < 0.05 \*\*0.001 ≤ p < 0.01.

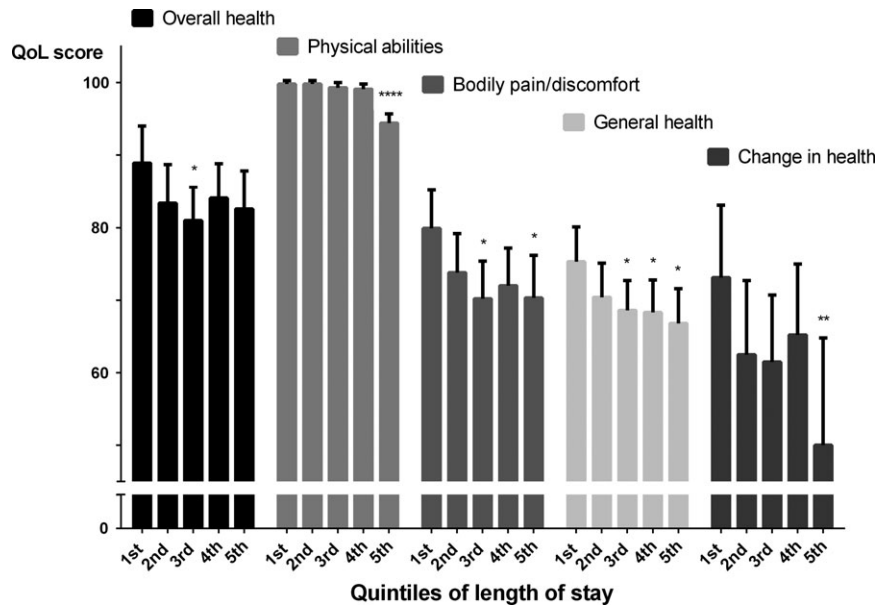
<sup>†</sup>1st quintile as reference category, regression coefficients of 2nd–5th quintiles.

<sup>‡</sup>We show only domains with results with p < 0.05.

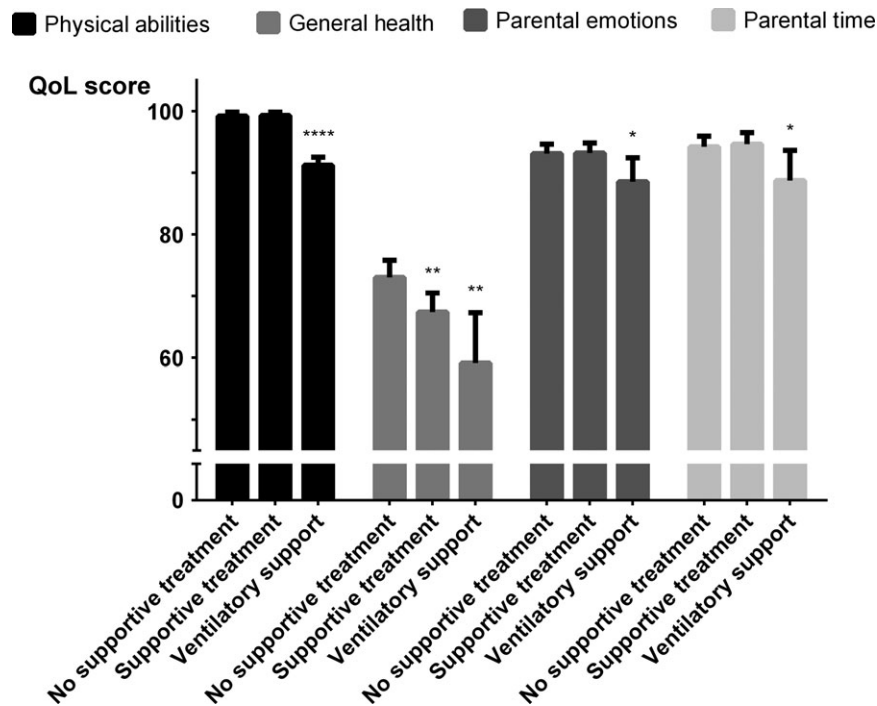
<sup>§</sup>95% CI in brackets.

<sup>¶</sup>Lower rows – adjusted for age, gender and one previous episode of obstruction.

<sup>¶¶</sup>Interaction with prematurity. Including this variable would have made the regression coefficient for the 5th quintile 15.3% more negative.



**Figure 1** Length of stay and QoL. Quality of life is given by domains with statistically significant associations with bronchiolitis severity based upon the quintiles of length of stay. Weighted mean scores are shown for each quintile. \* $p < 0.05$ . \*\* $0.001 < p < 0.01$  \*\*\*\* $p < 0.0001$ .



**Figure 2** Bronchiolitis severity and QoL. Quality of life is given by domains with statistically significant associations with bronchiolitis severity based upon the need for supportive care. Supportive care: Supplementary oxygen or nasogastric tube feeding, but not ventilatory support. Weighted mean scores are shown for each quintile. \* $p < 0.05$ . \*\* $0.001 < p < 0.01$  \*\*\*\* $p < 0.0001$ .

**Severity of bronchiolitis upon hospital admission**

For each increase in the clinical score points at inclusion, a significant reduction of 4.3% points (95% CI 1.9–6.8,  $p = 0.0007$ ) of QoL score in the discomfort and bodily pain domain, the only domain with significant findings in

this respect, was observed, after adjustment for age, gender and maternal ethnicity.

In analyses adjusted for age and gender, SpO2 was associated with the physical abilities domain. A significantly lower score was reported for infants with an SpO2 of

**Table 4** Significant associations between severity of acute bronchiolitis and quality of life domains<sup>†‡§</sup>, adjusted

QoL domain		No supportive treatment n = 104	Supportive treatment (nasogastric tube or O <sub>2</sub> ) n = 91	Ventilatory support n = 14
Physical abilities <sup>¶</sup>	Age and gender adjusted only	99.7 (99.4, 100.0)	-0.4 (-1.1, 0.3)	-7.3 (-8.9, -5.7) <sup>****</sup>
	Adjusted for one previous obstruction <sup>††</sup>	98.9 (97.8, 99.9)	-0.6 (-1.5, 0.3)	-6.2 (-8.1, -4.3) <sup>****</sup>
General health	Age and gender adjusted only	75.7 (71.2, 80.1)	-6.0 (-10.1, -1.9) <sup>**</sup>	-16.1 (-24.6, -7.5) <sup>***</sup>
	Adjusted for one previous obstruction	73.9 (68.9, 78.9)	-5.3 (-9.7, -0.9) <sup>*</sup>	-15.8 (-24.5, -7.0) <sup>***</sup>
Parental impact – emotions	Age and gender adjusted only	93.3 (91.9, 94.8)	0.1 (-2.1, 2.2)	-5.2 (-9.5, -0.9) <sup>*</sup>
	Adjusted for one previous obstruction	93.9 (91.4, 96.4)	0.0 (-2.2, 2.2)	-5.2 (-9.4, -1.0) <sup>*</sup>
Parental impact – time <sup>‡‡</sup>	Age and gender adjusted only	94.2 (92.4, 95.9)	0.3 (-2.1, 2.8)	-6.5 (-11.7, -1.2) <sup>*</sup>
	Adjusted for one previous obstruction	94.0 (90.9, 97.1)	0.4 (-2.3, 3.1)	-6.5 (-11.9, -1.0) <sup>*</sup>

\*0.01 ≤ p < 0.05 \*\*0.001 ≤ p < 0.01 \*\*\*0.0001 ≤ p < 0.001 \*\*\*\*p < 0.0001.

<sup>†</sup>Weighted means of no supportive treatment group as reference, regression coefficients of supportive treatment and ventilatory support groups.

<sup>‡</sup>We include only domains showing results with p < 0.05.

<sup>§</sup>95% CI in brackets.

<sup>¶</sup>Interaction with prematurity: Including prematurity in the model would have made the regression coefficient for ventilatory support 10.5% less negative.

<sup>††</sup>Lower rows – adjusted for age, gender and one previous episode of obstruction.

<sup>‡‡</sup>Interaction with prematurity: Including prematurity in the model would have made the regression coefficient for ventilatory support 8.9% more negative.

< 92%, with a mean difference of 6.7% points lower (95% CI 4.8–8.5), compared to the mean score reported for infants with an SpO<sub>2</sub> of > 92% of 99.8 (95% CI 99.4–100.2, p < 0.0001).

**DISCUSSION**

The infants' QoL nine months after hospitalisation for acute bronchiolitis was significantly associated with the severity of the acute illness, mostly in the domain of general health. Infants needing ventilatory support had the poorest QoL nine months later, with significant reductions in four of the 13 domains.

The present study shows, for the first time to our knowledge, that the severity of acute bronchiolitis in hospitalised infants had implications for their QoL almost a year later. We previously showed that, of the asthma risk factors, atopic eczema had the greatest impact on reduced QoL (16), whereas being hospitalised for acute bronchiolitis in infancy was significantly associated with later reduced QoL. The present study extends the previous observations, as it now shows that disease severity appeared to negatively influence the QoL of the infants and the parental perception of the child's health nine months later.

Increased LOS was associated with reduced quality of life in four of the 13 domains, including a considerably less favourable change in health score. Thus, LOS had a greater impact on the change in the health score nine months after hospitalisation than having a diagnosis of atopic dermatitis, as previously shown (16). The impact on QoL reduction by receiving supportive treatment other than ventilatory support was similar to the impact of atopic eczema (16). Similar to our study, a Dutch study in young children (15) showed that having had the respiratory syncytial virus (RSV) infection and a wheezing illness had a significant impact on the general health domain of the ITQOL. Within

this domain, our observed reduction of 16.1% points in infants receiving ventilatory support is similar to the 17% points reduction reported in infants with severe versus no asthma-like symptoms (19) and the 14.7% points reduction in four-year-old children with persistent wheezing compared to the reference group (25). On the other hand, a difference of 22.8% points was reported in infants and preschool children with wheeze and without wheeze (18). Collectively, the reported magnitude of QoL reductions in the presence or severity of respiratory disease is likely to be of clinical relevance.

Reductions in the QoL scores in four domains – physical abilities, general health, parental emotions and parental time – in infants receiving ventilatory support have, to our knowledge, not previously been demonstrated. Our results are in contrast to the study by Bont et al. (17), who did not find associations between mechanical ventilation and QoL two years later in 28 of 128 infants hospitalised with RSV infection. However, they used the TAPQOL questionnaire, assessing 13 scales including the stomach, skin, appetite and liveliness, and their QoL rating took place at three years of age compared to less than one year later, as in the present study. The differences in results may be related to different methods of measuring QoL and the time lapse between exposure and QoL measurement.

This study, and others, report variations in the associations between disease and the different QoL domains. As was found in the present study, reductions in the domain physical abilities were consistently or more strongly associated with several respiratory disease severity characteristics. We found that low SpO<sub>2</sub> at inclusion, or the need for ventilatory support, had a negative impact on this domain, in line with the impact of RSV infection on the domains physical abilities and general health reported in a Dutch study of young children with five health conditions (15). Similarly, a study of four-year-old children with persistent



wheeze showed an association between wheezing and a physical summary score (25). Young age, rendering the infants more prone to severe respiratory viral infections, is unlikely to explain the finding, as the association between ventilatory support and reduced physical abilities remained significant after adjustment for age at inclusion and gender in our study.

The finding that the severity score upon hospital admission was independently associated with later QoL has to our knowledge not previously been shown. Although this association was only found for bodily pain and discomfort, it may indicate that disease severity, already assessed at the time of hospital admission, may have an impact on later disease or health perception.

The present study may be limited by the relatively low rate of QoL answers from the original bronchiolitis group of the randomised controlled trial (52%), leading to an overrepresentation of parents with higher education and Caucasian ethnicity. On the other hand, we found no significant interaction or confounding by maternal education or ethnicity. The respondent and nonrespondent groups were similar in terms of most background characteristics, and the analyses that included maternal ethnicity and education did not reach the confounding criteria in the robust regression analyses. We therefore believe that the effect of selection bias on the associations was limited.

Due to lack of information, we were unable to assess possible influences of severe newborn disease. However, prematurity did not significantly confound the results, and few infants included in the study were expected to have had the need for mechanical ventilation around birth.

## CONCLUSION

The severity of acute bronchiolitis, assessed by length of stay in hospital, the need for supportive treatment and the disease severity at hospital admission were all associated with reduced quality of life nine months later. Infants receiving ventilatory support had the poorest QoL almost a year after the acute disease. Our results suggest that infants with severe acute bronchiolitis, judged by increased LOS or the need for supportive care, should be considered for clinical follow-up investigations and for possible management to prevent long-term health consequences.

## ACKNOWLEDGEMENTS

We thank all participants in the Bronchiolitis Study Group, as well as the several hundred study staff who were involved in recruitment and running of the study. These include, but are not limited to: Sabine Kristin Brüggmann-Pieper, Vestre Viken Hospital Trust, Ane Charlotte Brun, Vestfold Hospital Trust.

## CONFLICTS OF INTERESTS

None of the authors have any conflict of interests to report.

## FUNDING

The first author has a 50% fellowship from the Innlandet Hospital Trust's Research Fund.

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# Morning Salivary Cortisol in Young Children: Reference Values and the Effects of Age, Sex, and Acute Bronchiolitis

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**Objective** To identify morning salivary cortisol reference values in infancy and at 2 years of age and to investigate the influence of age, sex and acute bronchiolitis.

**Study design** In this South-East Norwegian cohort study, 308 children hospitalized with moderate to severe acute bronchiolitis in infancy in 2010-2011 were compared with 223 healthy controls included in 2012 by measuring morning salivary cortisol levels at inclusion and at 2 years of age. Samples were collected shortly after awakening after 6 AM. The influences of age, sex, and acute bronchiolitis were assessed by regression analysis.

**Results** In infancy, cortisol values were higher in acute bronchiolitis, with an age- and sex-adjusted weighted mean group difference of 13.9 nmol/L (95% CI 8.1-19.7;  $P < .0001$ ). The median level in reference group was 23.7 nmol/L (95% CI 9.7-119.6). At 2 years of age, sex but not inclusion groups differed, with significantly higher values in girls. The weighted mean of all boys' cortisol levels was 32.4 nmol/L, (95% CI 30.5-34.3), and all girls' levels were 36.9 nmol/L (95% CI 34.7-39.2;  $P < .003$ ).

**Conclusions** Salivary cortisol levels were higher at 2 years of age than in infancy in the reference group, were higher in girls than in boys at 2 years of age, and were higher in infants at the time of acute bronchiolitis than in healthy infants. (*J Pediatr* 2017;184:193-8).

**Trial registration** ClinicalTrials.gov: NCT00817466

Cortisol levels normally show a circadian rhythm with physiologically increased levels in the morning, with an additional cortisol awakening response.<sup>1,2</sup> Cortisol levels can be analyzed in blood, urine, and saliva.<sup>3-5</sup> Salivary samples are noninvasive, and do not induce the trauma, stress, and potentially higher cortisol compared with serum sampling.<sup>6</sup> However, a potential shortcoming of salivary measurement is the issue of spot sampling of a biomarker with known diurnal variation.<sup>7</sup> There are few reports on reference values of morning salivary cortisol levels in infants and toddlers, reflecting the biologically active, free fraction of serum cortisol.<sup>8-10</sup>

Reduced morning cortisol has been associated with allergic diseases such as asthma and allergic rhinitis in young and older children, pointing to an involvement of adrenocortical function.<sup>11-14</sup>

Links between stress, cortisol levels, and asthma in early childhood or later asthma development have been proposed,<sup>14-16</sup> and we recently showed that being hospitalized for acute bronchiolitis in infancy increased the risk for reduced health-related quality of life.<sup>17</sup> However, investigation of possible causal associations between infant salivary cortisol levels and later asthma requires relevant reference values of morning salivary cortisol levels in early childhood.

Our primary aim was to describe reference values for morning salivary cortisol levels during infancy and at 2 years of age. Second, we sought to investigate whether age, sex, or acute moderate to severe bronchiolitis in infancy influenced morning salivary cortisol levels.

## Methods

The present study included 531 children with at least 1 (total 762) salivary cortisol level measurement in infancy, when they were recruited into the study and/or at the 2-year follow-up (Figure 1; available at [www.jpeds.com](http://www.jpeds.com)). The source population included 404 infants hospitalized with moderate to severe acute bronchiolitis in 8 pediatric departments of southeast Norway. Additionally, 240 infants were recruited by postal invitation to 3000 randomly selected children 0-12 months of age from the municipalities of Oslo and Fredrikstad<sup>17</sup> from March 22, 2012 to July 2, 2012, who were included in the Bronchiolitis ALL SE-Norway study

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L.R. is employed as a fellow paid by the Innlandet Hospital Trust's Research Fund (150189), which also refunded the costs for the salivary cortisol analyses. The authors declare no conflicts of interest.

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<http://dx.doi.org/10.1016/j.jpeds.2017.01.064>

RSV Respiratory syncytial virus

(hereafter referred to as the Bronchiolitis study).<sup>18</sup> As previously reported, respiratory syncytial virus (RSV) was identified in 83% and human rhinovirus in 34% of participants; 44% received oxygen therapy and 7.4% received ventilatory support.<sup>18,19</sup> The follow-up investigation at 2 years, performed from September 27, 2011, to December 14, 2011, September 11, 2012 to December 18, 2012, and October 7, 2013 to January 22, 2014, was attended by 499 of the initial 644 infants (77.5%).

Inclusion criteria for all infants were age 0-12 months, and for inclusion into the Bronchiolitis study, moderate to severe bronchiolitis, with a clinical score of at least 4 on a scale from 0 to 10 (most severe).<sup>18</sup> Exclusion criteria for all infants were severe underlying disease, and for the Bronchiolitis study, more than one episode of either bronchopulmonary obstruction or cough lasting for longer than 4 weeks before recruitment.

### Procedures

Clinical investigations, morning cortisol sampling, and parental structured interviews were conducted at inclusion and at 2 years of age.

The study was approved by the Regional Committee for Medical and Health Research Ethics and The Norwegian Data Protection Authority and was registered in the Norwegian bio bank registry as well as [ClinicalTrials.gov](http://ClinicalTrials.gov) number, NCT00817466. Written informed consent was obtained from caregivers of all children.

### Saliva Sampling

Parents were instructed to sample saliva in the morning as soon as possible after the child's awakening (after 6:00 a.m.) and before the children's first meal. Two small (0.7 × 1.8 mm), tasteless, arrowhead-shaped Sorbette (hydrocellulose, Salimetrics Europe Ltd, Suffolk, UK) microsponges attached to plastic applicator shafts were inserted into the child's mouth, preferably under the tongue, and kept there for a total of 60-90 seconds, until the microsponges were soaked with saliva.<sup>20</sup> The salivary samples in their respective standard containers were brought to the investigation site, and thereafter frozen at -86°C until transferal to Karolinska Institutet, Stockholm, for analysis. Radioimmunoassay was performed according to the manufacturer's instructions using kits from Cisbio Bioassays (Codolet, France) with monoclonal rabbit antibodies binding cortisol. For further description, see the [Appendix](#) (available at [www.jpeds.com](http://www.jpeds.com)). The assay is standardized against the reference method, mass spectrometry.

### Main Outcome

Reference values were defined as salivary cortisol levels (nmol/L) ranging from the 5th to the 95th percentile in infancy (at inclusion) and at 2 years of age. For comparison with other studies, geometric means were estimated and reported. Secondary outcomes for assessing potential influence of age, sex, and acute bronchiolitis were weighted mean salivary cortisol levels (nmol/L) with 95% CI.

### Statistical Analyses

Background characteristics are given as means with SD, mean with minimum and maximum, or numbers with percentages, as appropriate. Neither morning salivary cortisol levels nor their natural logarithms were normally distributed. Percentiles including 95% CI for the 5th and 95th percentiles were used for estimating reference values.

To assess the potential impact of age, sex, and hospitalization for acute bronchiolitis on morning salivary cortisol levels, associations with cortisol were examined by the Huber M method of regression analysis,<sup>21</sup> whereas associations between dichotomous variables were analyzed by Pearson  $\chi^2$  test. Weighted means were calculated by Huber M regression methods, applying groups as categorical values, and estimating intercepts as the weighted mean. The significance level was set at .05. Interaction between age, sex, and morning salivary cortisol was tested by 2-way robust regression. Percentile analyses and robust regression analyses were done with NCSS 2007 (NCSS Statistical Software, Kaysville, Utah); otherwise, IBM SPSS (SPSS Inc, Chicago, Illinois) version 22.

## Results

Salivary samples were obtained from January 15, 2010, to May 20, 2011, from 383 infants at a mean age of 5.1 months (range 0.2-13.4) and from 379 children at a mean age of 24.2 months (range 17.2-34.7; [Table I](#)), with samples at both time points in 231 children and on 1 occasion in the remaining 300 children ([Figure 1](#)). Background characteristics were similar between children from the reference group and bronchiolitis group with respect to sex, age at 2 years, parental asthma, ethnicity, and breast feeding, but significantly different with respect to weight and length, parental education, and use of inhaled corticosteroids ([Table I](#)). No interaction was found between age, sex, or morning salivary cortisol at the 2 time points.

Morning salivary cortisol ranged from 1.9 to 691.4 nmol/L in infancy and from 2.5 to 189.0 nmol/L at 2 years of age.

### Reference Values

In infancy, the reference group had a geometric mean of 26.8 (95% CI 24.0-30.0) nmol/L with the reference values given by percentiles ([Table II](#)). The bronchiolitis group had significantly higher cortisol values ([Figures 2-4](#); [Figures 2](#) and [3](#) available at [www.jpeds.com](http://www.jpeds.com)), with a geometric mean of 37.0 nmol/L (95% CI 33.0-41.4) and a median of 39.9 nmol/L.

At 2 years of age, the weighted mean cortisol values were similar in the control and bronchiolitis groups. Reference values were therefore based on values including all children ([Table II](#); [Figures 5](#) and [6](#), available at [www.jpeds.com](http://www.jpeds.com)), with a geometric mean of 32.1 nmol/L (95% CI 30.4-33.9).

Cortisol levels were above 3 SD in 1.5% and in 1.9% of the children at inclusion and at 2 years of age, respectively. By robust regression, we found no association or individual

**Table I. Background characteristics of the 531 children with cortisol results during infancy, at 2 years of age, at either or both times**

	Reference group	Bronchiolitis group	P value
All subjects (n = 531)	223	308	
Boys, n (%)	125 (56.1%)	186 (60.4%)	.317
Age (mo), mean (min-max)			
Inclusion (n = 531)	6.5 (1.0-13.4)	4.1 (0.2-11.9)	<.001
Two years of age (n = 453)	24.2 (17.2-34.7)	24.3 (18.8-34.7)	.667
Time (mo) between visit 1 and 2 (n = 379)	17.7 (1.2)	20.0 (1.2)	<.001
Weight (kg), mean (SD)			
Inclusion (n = 518)	7.9 (1.8)	6.5 (1.8)	<.001
Two years of age (n = 433)	12.9 (1.6)	13.2 (1.7)	.05
Length (cm), mean (SD)			
Inclusion (n = 371)	67.8 (6.4)	62.9 (6.8)	<.001
Two years of age (n = 434)	88.6 (4.4)	87.1 (4.1)	<.001
Parental asthma (n = 520), n (%)	86 (29.6%)	94 (31.6%)	.632
Parental allergic rhinitis (n = 527), n (%)	84 (37.7%)	89 (29.3%)	.049
High education, n (%)*			
Mothers (n = 483)	199 (89.2%)	71 (63.3%)	<.001
Fathers (n = 487)	179 (81.4%)	150 (56.2%)	<.001
Caucasian, n (%)			
Mothers (n = 495)	211 (94.6%)	254 (93.4%)	.566
Fathers (n = 491)	206 (92.4%)	252 (94.0%)	.466
Breastfed, n (%)			
Inclusion (n = 453)	148 (75.1%)	187 (73.0%)	.617
Two years of age (n = 439)	10 (5.4%)	7 (2.8%)	.161
Second-hand smoke, n (%)			
Inclusion (n = 485)	9 (4.1%)	44 (16.5%)	<.001
Two years of age (n = 446)	1 (0.5%)	6 (2.4%)	.124
Boys with asthma at 2 years of age	5 (4.7%)	39 (22.9%)	<.001
Girls with asthma at 2 years of age	3 (3.6%)	13 (11.6%)	.042
Current inhaled corticosteroids at 2 years of age (n = 453)	9 (4.6%)	46 (18.0%)	<.001
Gestational age at birth < 37 <sup>0/7</sup> weeks (n = 443)	8 (3.8%)	28 (11.9%)	.002

\*Defined as completed at least 3 years of schools after secondary school.

repeatability between salivary cortisol at inclusion and at 2 years of age in any of the groups, with a regression coefficient of 0.001 ( $P = .93$ ) in the reference group and a regression coefficient of  $-0.010$  ( $P = .83$ ) in the bronchiolitis group.

**The Role of Age and Sex**

The weighted mean cortisol in the reference group was significantly higher at age 2 years (34.9 nmol/L; 95% CI 32.6-37.2) than in infancy (28.7 nmol/L; 95% CI 25.1-32.4) with a difference of 7.8 nmol/L (95% CI 2.4-13.1;  $P = .004$ ),

whereas the reverse was found in the bronchiolitis group at age 2 years (33.8 nmol/L; 95% CI 31.8-35.7) vs infancy (41.5 nmol/L; 95% CI 37.6-45.4), with a difference of  $-6.4$  nmol/L (95% CI  $-11.0, -1.8$ ;  $P = .006$ ). Sex-stratified results are shown in **Figures 3** and **6**.

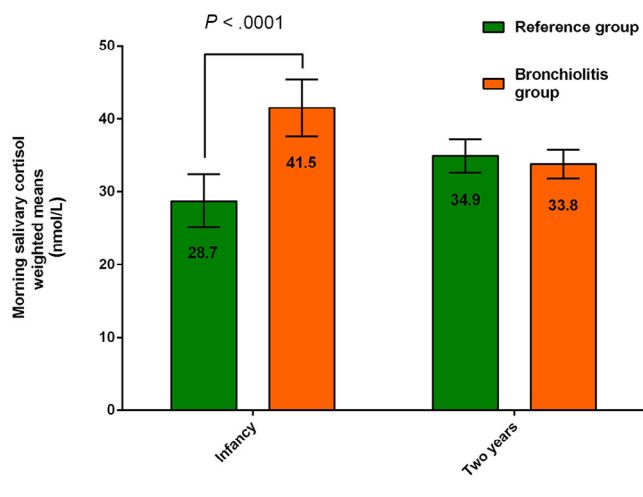
However, increasing age within infancy or at 2 years of age was not associated with cortisol levels in regression analyses (**Table III**; available at [www.jpeds.com](http://www.jpeds.com)).

At 2 years of age (but not in infancy), girls had significantly higher weighted mean and geometric mean morning salivary cortisol compared with boys (**Figures 6** and **7**

**Table II. Reference values: morning salivary cortisol percentile levels (nmol/L) at inclusion\***

	Percentiles								
	2.5	5	10	25	50	75	90	95	97.5
Inclusion									
Reference	4.5	9.7	12.5	18.2	23.7	40.1	58.3	119.6	222.1
95% CI		3.1-12.1						64.9-258.6	
Two years									
All	11.8	13.6	16.2	24.6	34.1	43.9	56.6	65.2	80.2
95% CI		12.1-15.2						60.5-73.1	
Boys	9.0	12.7	15.5	23.7	31.8	42.2	51.7	62.8	74.8
95% CI		8.3-14.8						55.6-77.6	
Girls	12.9	14.4	16.9	26.7	38.5	45.6	60.3	69.7	112.0
95% CI		12.6-16.5						62.0-162.1	

\*From the 199 infants in the reference group (0-13 months of age), and at the 2-year follow-up from all 379 children, stratified by sex (boys n = 220, girls n = 159).



**Figure 4.** Comparison of mean salivary cortisol levels, control and bronchiolitis groups. Morning salivary cortisol (weighted mean [95% CI]) levels given for children in the reference group and bronchiolitis group at inclusion and at 2 years of age. The cortisol levels in infants with acute bronchiolitis were significantly higher than levels in the reference group in infancy and the bronchiolitis follow-up group at 2 years ( $P < .0001$ ). Weighted mean within the bars.

[available at [www.jpeds.com](http://www.jpeds.com)]; weighted mean 36.9 nmol/L [95% CI 34.7-39.2] vs 32.4 nmol/L [95% CI 30.5-34.3] and geometric mean of 35.1 nmol/L [95% CI 32.4-38.0] vs 30.1 nmol/L [95% CI 28.0-32.3], respectively;  $P < .003$ ). Reference values are therefore presented stratified for sex (Table II). The sex difference in cortisol levels was not associated with the higher frequency of a doctor's diagnosis of asthma in boys (results not shown).

### The Role of Acute Moderate-to-Severe Bronchiolitis

Morning salivary cortisol levels were significantly higher in infants with acute bronchiolitis than in infants in the reference group (Figures 4 and 6, with a weighted mean difference of 12.8 nmol/L [95% CI 7.4-18.1] that remained significant after adjusting for age and sex (13.9 nmol/L [95% CI 8.1, 19.7];  $P < .0001$ ).

At 2 years of age, however, the weighted mean cortisol levels were similar in the bronchiolitis (33.8 nmol/L; 95% CI 31.8-35.7) and reference group (34.9 nmol/L; 95% CI 32.6-37.2), and are thus presented for all 379 children (34.3 nmol/L; 95% CI 32.8-35.8).

The predictability of cortisol at inclusion for assignment to the bronchiolitis or reference group is illustrated by a receiver operating characteristic diagram (Figure 8). The area under the curve was 0.634 (95% CI 0.577-0.690). The cutoff value of cortisol giving the highest sum of sensitivity and specificity is 36.9 nmol/L, giving a sensitivity of 51.6% and a specificity of 68.3%.

Neither breastfeeding, parental education, second-hand smoke, weight, nor length was associated significantly with

salivary cortisol level at inclusion or at 2 years of age (results not shown). Subgroup analyses of non-Caucasian vs Caucasian parents could not be performed owing to low numbers of non-Caucasian parents.

## Discussion

Reference values based on the 5th-95th percentiles were estimated in 199 children from a general population in infancy, and at 2 years of age in 379 children including the same reference population as well as children who in infancy were hospitalized with acute bronchiolitis. Salivary cortisol levels were significantly higher at 2 years of age than in infancy in the reference group, were significantly higher in infants with bronchiolitis compared with the reference group, and in girls at 2 years compared with boys. However, age was not associated significantly with morning salivary cortisol at either of the 2 time points.

We found generally higher values than salivary cortisol levels reported as quartiles by Ivars et al<sup>10</sup> in a Swedish infant population. Although our median value of 23.7 nmol/L (at a mean age of 6 months) was similar to the mean value of 24.6 nmol/L at 6 months of age reported by Ivars et al, their median cortisol levels varied from 5.1 to 10.9 nmol/L during infancy. Similar to our reference group, the Swedish study recruited 130 infants from a general population, with 95-120 samples collected each month through infancy, and both studies using the same radioimmunoassay method.<sup>10,22</sup> The differences in cortisol levels may be related to the timing of salivary sampling. In our study, parents sampled saliva as soon as possible after first awakening after 6 AM, before feeding, in contrast with the Swedish parents who collected saliva samples at least 1 hour after solid food, sleep, or crying and riding a car, and 30 minutes after intake of liquids. In line with a circadian rhythm, Ivars et al observed that early morning samples (between 7:30 and 9:30 a.m.) were higher than evening samples (between 6:30 and 9:30 p.m.).<sup>10</sup> Using the same analysis protocol, values between those found in the present study, and those found by Ivars et al were reported by Stenius et al<sup>23</sup> in 6-month-old infants (geometric mean of 14.9 nmol/L) with salivary sampling within one-quarter of an hour after awakening in the morning, and before the first meal. Higher cortisol levels in samples taken as soon as possible after awakening are supported by the decreasing cortisol levels observed in samples taken hourly from 8 to 10 a.m. in children older than 2 years of age participating in a Japanese study of 57 healthy 0.5 to 4.0-year-old children.<sup>9</sup> Compared with the higher reference values observed in the present study, their defined lowest and upper limits of the reference range in micrograms/dL—0.076 (equals 2.1 nmol/L) and 0.827 (equals 22.8 nmol/L), respectively—may reflect that the samples were taken later during the day. An awakening cortisol response, described as the period of cortisol secretory activity in the immediate 45-60 minutes after awakening,<sup>24</sup> reaches a maximum about 30 minutes after wakefulness.<sup>1</sup> An awakening response is supported by a study in infants at a mean age of 2 months, reporting a mean value of 0.31  $\mu\text{g/dL}$  (8.6 nmol/L) immediately after awakening and



0.60  $\mu\text{g}/\text{dL}$  (16.6 nmol/L) 30 minutes after awakening from at least 30 minutes of sleep.<sup>25</sup> Thus circadian rhythm, with highest peaks in the morning, as well as awakening response, as described by Michels et al<sup>26</sup> in only 52% of children 5-11 years of age, may explain the higher values in the present study.

The higher cortisol values among girls at 2 years of age in our study is a novel finding, and may have several causes. Higher cortisol levels in girls may be related to a more pronounced cortisol awakening response compared with boys, as described by Pruessner et al<sup>27</sup> in 12 year-old children, or may be explained partially by a prolonged “mini-puberty” in girls compared with boys.<sup>28</sup> Because salivary cortisol reflects the free fraction of cortisol, our finding cannot be explained by a higher level of corticosteroid-binding globulin. Similarly, sex differences in cortisol levels could not be explained by the higher frequency of a doctor’s diagnosis of asthma among boys.

Higher morning salivary cortisol in infants with acute bronchiolitis suggests possible pathophysiological involvement of cortisol in acute bronchiolitis, although cortisol levels could not be used to classify infants into bronchiolitis or control groups owing to the overlapping values, as illustrated by the receiver operating characteristic curve and dot plots. The infants had their first samples taken during moderate to severe acute bronchiolitis,<sup>18</sup> in line with higher cortisol values found with severe disease that possibly reflects suppression of the Th1 response, as described in RSV infection in infants.<sup>29</sup>

A possible limitation of our study was the skewed study population, where approximately 89% of mothers in the reference group and 63% in the bronchiolitis group had higher education compared with the national average of 48% of all women between 25 and 50 years of age, according to Statistics Norway.<sup>30</sup> We found no association between parental education and salivary cortisol levels. Another potential limitation is the low proportion of successful collection of samples at both study points, especially in the bronchiolitis group. However, the high probability of a difference between ill and control groups at inclusion and between the sexes at the follow-up when both groups are analyzed together makes it likely that they reflect true differences.

Owing to the high proportion of infants with detected RSV during acute bronchiolitis, it was not possible to perform robust analyses into the potential specific impact of RSV on salivary cortisol level.

A potential shortcoming of salivary measurement is the representativeness of a spot sample of a biomarker with known diurnal variation.<sup>7</sup> However, measuring morning salivary cortisol may provide information about the hypothalamic-pituitary-adrenal axis and capacity for reaching high peak values that may be blunted by measuring a 24-hour or several hour urinary value. Another potential source of variation, particularly for the highest values, could be blood contamination. However, other studies have shown that blood contamination has little impact on salivary cortisol measurements.<sup>31,32</sup>

Greater variations at the upper end of salivary vs serum cortisol levels may be explained by the fact that salivary cortisol

is an ultrafiltrate of the free fraction of serum cortisol. If acute stress or other factors lead to a surge of cortisol exceeding the corticosteroid globulin binding capacity, it is possible that we may find a higher relative increase in salivary vs total plasma cortisol.<sup>33</sup>

Sampling time is likely to be crucial for salivary measurements. We standardized the sampling to the best of our ability, but cannot rule out potential deviations from the time of sampling. However, our approach of asking parents to sample as soon as possible after awakening reduces potential variation in time. Also, we did only 1 sampling per child on each occasion; thus, repeatability could not be assessed. However, Nagakura et al<sup>9</sup> found no significant day-to-day variation between 3 samples taken at 4- to 8-day intervals.

Reference morning cortisol values in infants were 9.7 to 119.6 nmol/L (5th-95th percentile), compared with 11.8 to 80.2 nmol/L at 2 years. Sex-specific reference values may be necessary, because girls at 2 years of age in the present study had higher cortisol values than boys. Acute bronchiolitis in infancy was associated with higher morning salivary values during hospitalization, but seemed not to influence morning cortisol levels at 2 years of age. ■

*We thank all children and caregivers, participants in the Bronchiolitis Study Group, the several hundred hospital staff that were involved in recruiting patients and running the study, and involved members of the Oslo Research group of Asthma, Allergy in Children, the Lung and Environment (ORAACLE).*

Submitted for publication Sep 4, 2016; last revision received Jan 9, 2017; accepted Jan 26, 2017

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**Appendix**

**Methods:** In each tube was a known amount of <sup>125</sup>I-labeled cortisol in Tris buffer, competing with the salivary cortisol to be attached to antibody binding sites. The saliva was kept within the tubes in a water bath at 37°C for 30 minutes before it was poured out. Then, the tubes were rinsed with a predefined amount of water and left upside down for a period until they were put into a gamma counter. A standard curve for

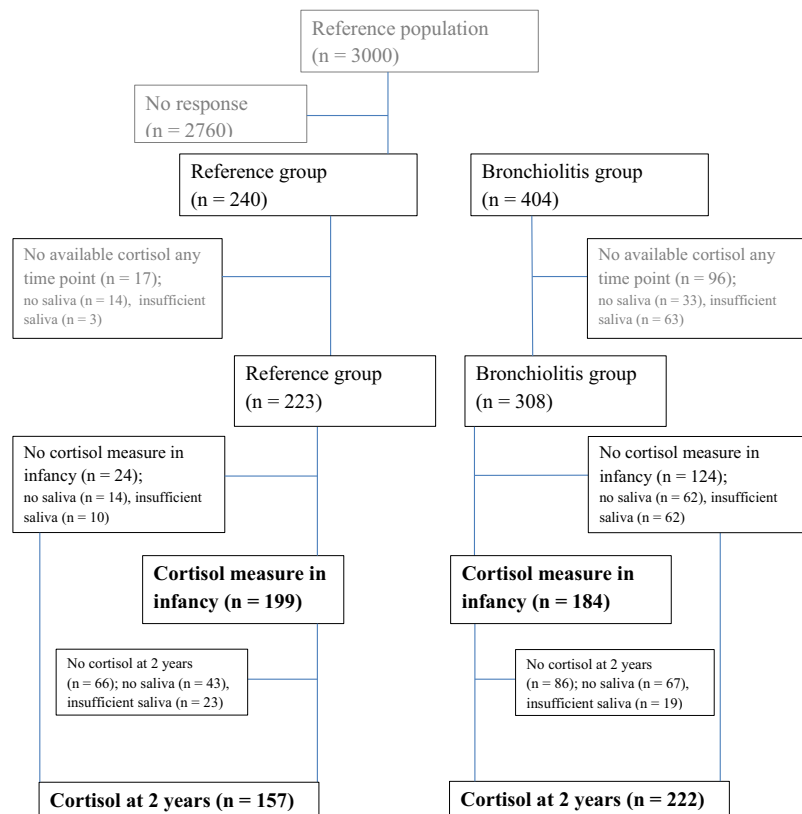
comparison was produced from a cortisol calibrating sample in the kit. The working range for the method is 0-2000 nmol/L and 150 μL of saliva is required. The analytical sensitivity is 3.0 nmol/L.

**Results:** At 2 years of age, the geometric mean values of morning salivary cortisol were 31.6 nmol/L (95% CI 29.5-33.9) in the bronchiolitis group and 32.8 nmol/L (95% CI 30.1-35.6) in the reference group.

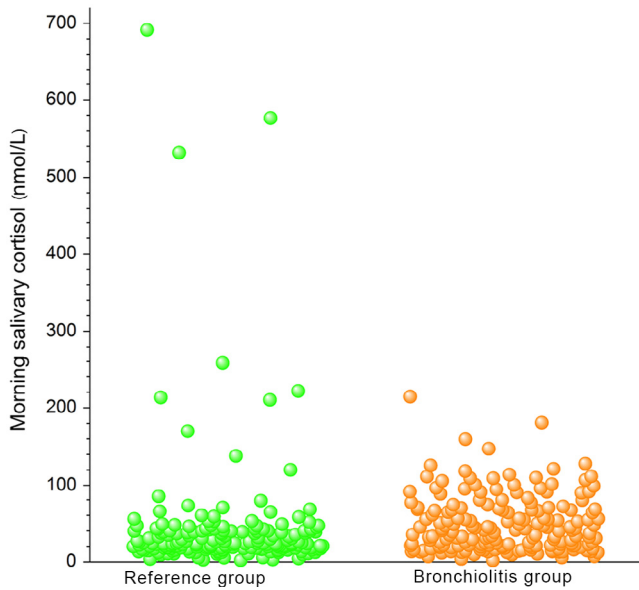
**Table III. Morning salivary cortisol (nmol/L)**

Cortisol levels	Reference group		Bronchiolitis group	
	Unadjusted weighted mean	Age increase	Unadjusted weighted mean	Age increase
Infancy	28.7	0.7	41.5	-0.6
95% CI	25.1 to 32.4	-0.2 to 1.6	37.6 to 45.4	2.4 to 1.2
Two years	34.9	0.0	33.8	-0.4
95 % CI	32.6 to 37.2	-0.6 to 0.6	31.8 to 35.7	-1.0 to 0.3

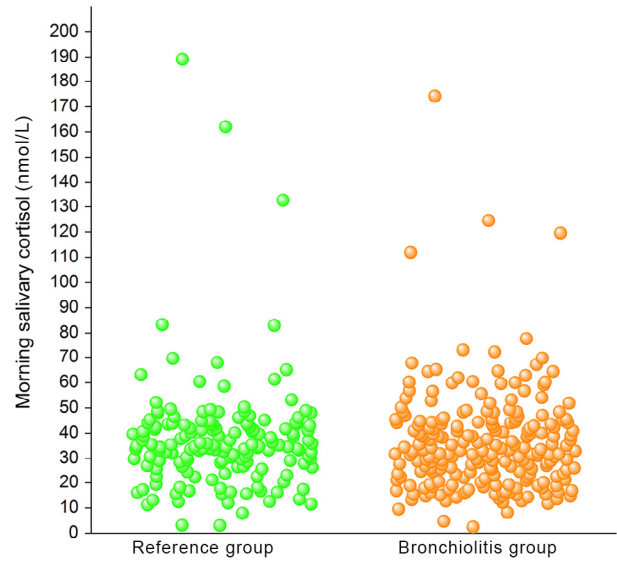
\*Given as unadjusted weighted mean for reference group and bronchiolitis group, sexes together, in infancy and at 2 years of age and changes by monthly increases in age at salivary cortisol sampling.



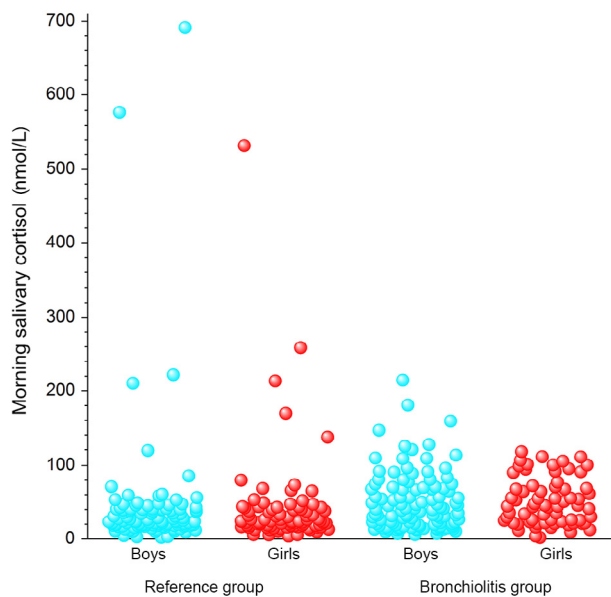
**Figure 1.** Flowchart of inclusion. Entrance of subjects with available cortisol measurements.



**Figure 2.** Morning salivary cortisol at inclusion, dot plot; each dot representing an individual.

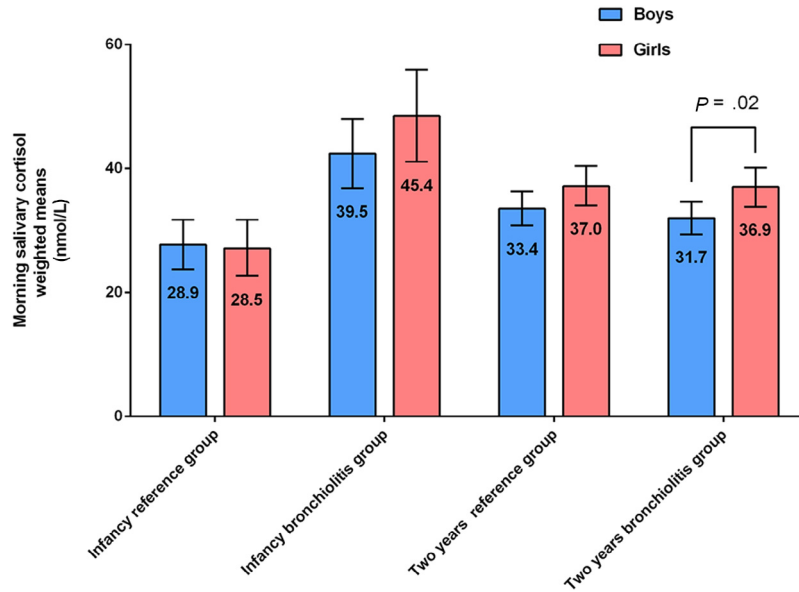


**Figure 5.** Morning salivary cortisol at 2 years of age, dot plot; each dot representing an individual.

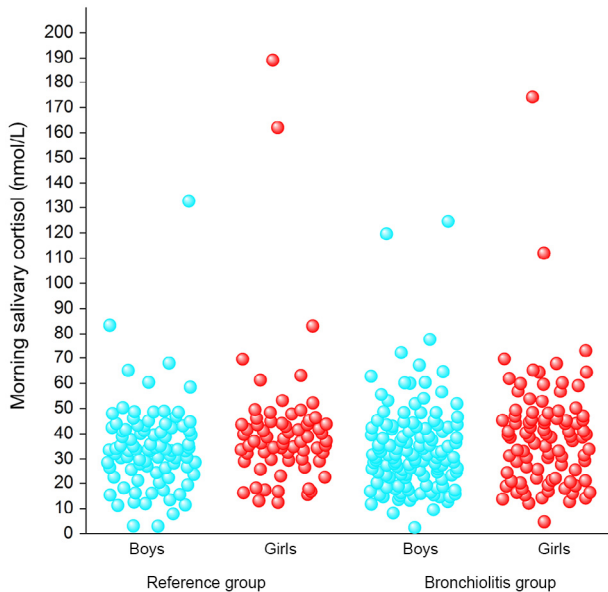


**Figure 3.** Sex distribution, morning salivary cortisol at inclusion dot plot; each dot representing an individual.

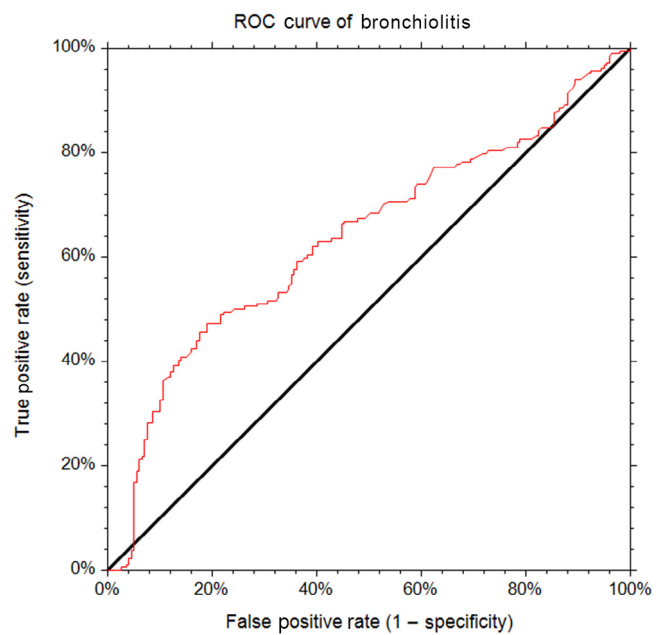




**Figure 6.** Morning salivary cortisol (weighted mean (95% CI)) are given for boys (blue bars) and girls (red bars) in infancy and at 2 years of age. P-value is shown for difference between sexes when <0.05. Weighted mean are shown within the bars. Cortisol levels were significantly higher for girls at 2 years of age.



**Figure 7.** Sex distribution, morning salivary cortisol at 2 years of age, dot plot; each dot representing an individual.



**Figure 8.** Receiver operating characteristic (ROC) curve, showing predictability for bronchiolitis by morning salivary cortisol at inclusion.







## RESEARCH ARTICLE

## Quality of life, salivary cortisol and atopic diseases in young children

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## Abstract

## Background

Children with atopic disease may have reduced health-related quality of life (QoL) and morning cortisol. Possible links between QoL, morning cortisol and atopic disease are unclear.

We aimed to determine if QoL was associated with morning salivary cortisol at two years of age, and if asthma, atopic dermatitis and/or allergic sensitisation influenced this association. Secondly, we aimed to determine if QoL at one year of age was associated with salivary cortisol one year later.

## Methods and findings

The Bronchiolitis All SE-Norway study included infants during hospitalisation for acute bronchiolitis in infancy (bronchiolitis group) and population-based control infants (controls). The present study included all 358 subjects with available Infant Toddler Quality of Life Questionnaire (ITQOL) from parents, consisting of 13 domains and morning salivary cortisol at two years of age. Answers from the same 0–100 score questionnaire, with optimal score 100 nine months after enrolment, was also available for 289 of these children at about one year of age. Recurrent bronchial obstruction was used as an asthma proxy. Atopic dermatitis was defined by Hanifin and Rajka criteria and allergic sensitisation by a positive skin prick test. Due to different inclusion criteria, we tested possible interactions with affiliation groups. Associations between QoL and cortisol were analysed by multivariate analyses, stratified by bronchiolitis and control groups due to interaction from affiliation grouping on results. At two years of age, QoL decreased significantly with decreasing cortisol in 8/13 QoL domains in the bronchiolitis group, but only with General health in the controls. The associations in the bronchiolitis group showed 0.06–0.19 percentage points changes per nmol/L cortisol for each of the eight domains ( $p$ -values 0.0001–0.034). The associations remained significant but diminished by independently including recurrent bronchial obstruction and atopic dermatitis, but remained unchanged by allergic sensitisation. In the bronchiolitis group only, 7/13 age and gender adjusted QoL domains in one-year old children were lower with lower cortisol levels at two years of age ( $p = 0.0005$ –0.04).

## OPEN ACCESS

**Citation:** Rolfsjord LB, Skjerven HO, Bakkeheim E, Berents TL, Carlsen K-H, Carlsen KCL (2019) Quality of life, salivary cortisol and atopic diseases in young children. PLoS ONE 14(8): e0214040. <https://doi.org/10.1371/journal.pone.0214040>

**Editor:** Maria Christine Magnus, Norwegian Institute of Public Health, NORWAY

**Received:** March 4, 2019

**Accepted:** July 23, 2019

**Published:** August 30, 2019

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**Data Availability Statement:** The data are stored in a research server at the Oslo University Hospital. The data are also used for ongoing works and studies. At the start of the study, consent was not given from the children's parents for open access to data for third parties. Therefore, they are not generally available. For requests for data access – please contact Head of the Department of paediatric allergy and pulmonology, Division of Paediatric and Adolescent Medicine, Oslo University Hospital, dr. Iren Matthews, P.O. Box 4956 Nydalen, NO-0424 OSLO, Norway, tel. no. +47 23 07 00 00.

**Funding:** The project was supported by a grant to the first author's employer, Innlandet Hospital Trust, (Project no. 150189 to LBR) from a research fund foundation, Klosterstiftelsen. Klosterstiftelsen does not have any specific URL, but further information can be reached at <https://www.purehelp.no/m/company/details/klosterstiftelsen/982953146>. No commercial companies funded the study or authors. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The other authors did not receive any specific funding for this work.

**Competing interests:** The authors have declared that no competing interests exist.

**Abbreviations:** AD, Atopic dermatitis; ITQOL, The Infant Toddler Quality of Life Questionnaire; QoL, Health related quality of life.

## Conclusions

At two years, most QoL domains decreased with lower salivary cortisol among children who had been hospitalised for acute bronchiolitis in infancy, but for one domain only among controls. Recurrent bronchial obstruction and to a lesser extent atopic dermatitis, weakened these associations that nevertheless remained significant. After bronchiolitis, lower QoL in one-year old children was associated with lower salivary cortisol at two years.

## Introduction

Development of asthma has been associated with acute bronchiolitis [1, 2] and asthma with reduced basal morning salivary cortisol, also in children without current use of inhaled corticosteroids [3]. Asthma [4, 5], atopic dermatitis [6], previous hospitalisation for acute bronchiolitis [7, 8], and psychological and physical stress [9, 10] have been associated with reduced health-related quality of life (QoL). Subjects exposed to pre- or postnatal stress may have a lower cortisol after exposure to acute stress if atopic as opposed to non-atopic subjects with the same exposures tending to have higher cortisol [11].

The generic, parent-based Infant Toddler Quality of Life Questionnaire (ITQOL-97) has shown reduced QoL in young children with obstructive airways disease [12], AD [8] and some other diseases [13]. Only five of the concepts or domains of ITQOL-97 specify a time period, each of past four weeks. In comparison, in children, chronic cough is increasingly defined as having lasted for more than four weeks ([14]. Thus, ITQOL-97 may possibly be sensitive to chronic disease and stress.

In the Bronchiolitis All SE-Norway study, we collected extensive information about the participants, including history and signs of atopic disease [15–17]. In the present add-on exploratory study, we applied the same survey of parent-reported QoL of the children and concepts of impact on the parents, i.e. ITQOL-97, at two time points. Based on published studies on morning or acute stress-induced cortisol [18–20], we hypothesised that *low* cortisol levels in periods without illness requiring acute hospitalisation may contribute to development of asthma. We further hypothesised that reduced QoL some months after severe acute illness in early life may be a marker of chronic stress, with subsequent lower future salivary cortisol levels.

We therefore primarily aimed to determine if QoL was associated with morning salivary cortisol at two years of age, and if asthma, atopic dermatitis and/or allergic sensitisation modified this association. Secondly, we aimed to determine if QoL at one year of age was associated with salivary cortisol at two years.

## Materials and methods

### Study design

From the source population of 644 children included in the Bronchiolitis ALL SE-Norway study enrolling infants who were hospitalised for acute bronchiolitis and controls recruited from a general population [8], we included all 358 children with available salivary cortisol and QoL at 24 months of age. The bronchiolitis group consisted of 203 infants with moderate to severe acute bronchiolitis at inclusion, and 155 were controls. For details, see Fig 1 and Supporting Information.

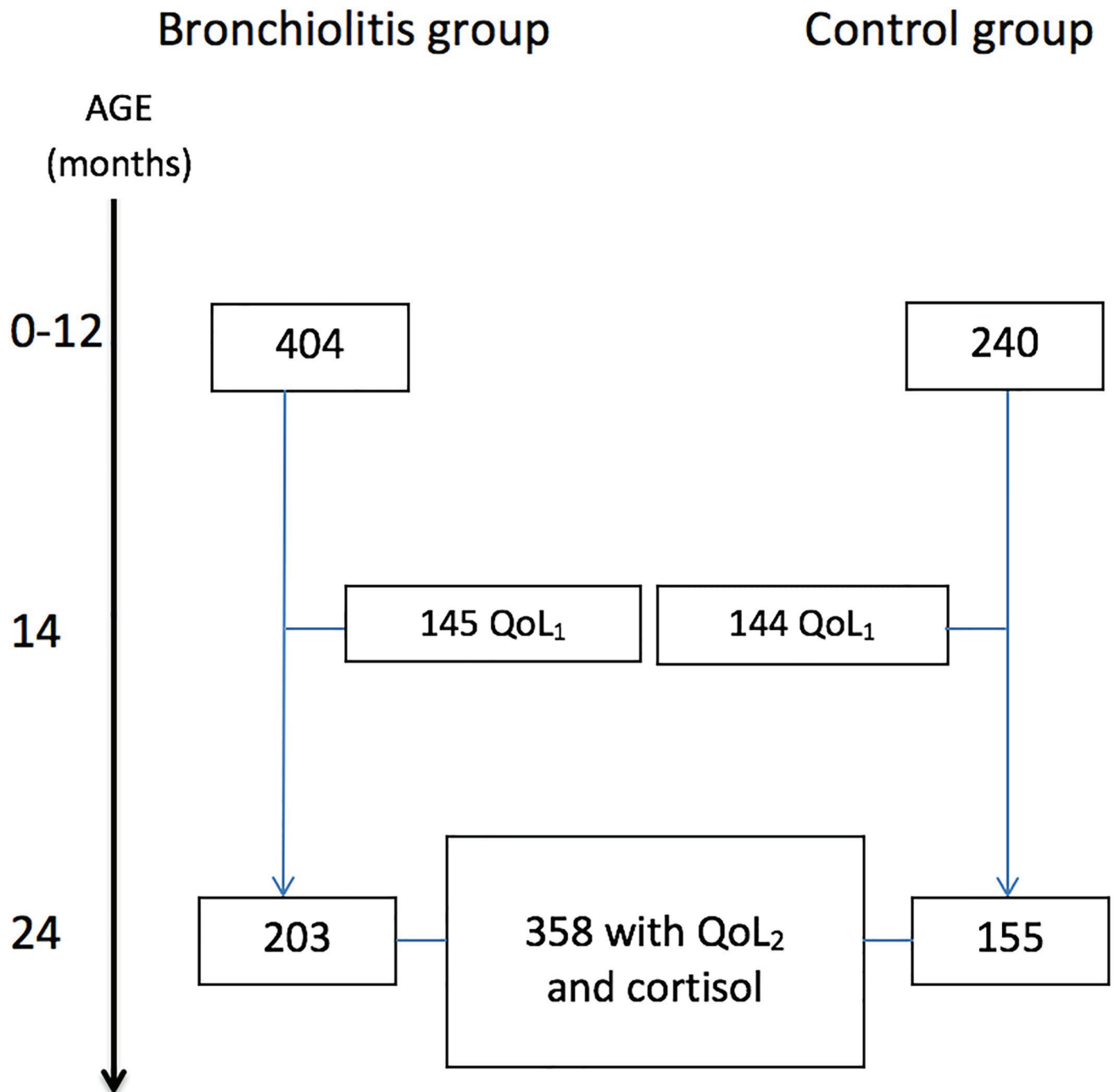


Fig 1. The Fig outlines the number of infants enrolled in the Bronchiolitis All SE-Norway study (top, n = 644) who were subsequently included in the present study (n = 358) for analyses based upon available Quality of life (QoL) and/or salivary morning cortisol at 24 months of age. The QoL questionnaires were completed nine months after enrolment at approximately 14 months of age (QoL<sub>1</sub>) as well as at the time of the clinical examination at 24 months of age (QoL<sub>2</sub>).

<https://doi.org/10.1371/journal.pone.0214040.g001>

Investigations at enrolment and at two years of age included clinical assessment, structured parental interviews and morning salivary sampling for cortisol. Skin prick test (SPT) for common inhalant and food allergens was performed at two years. Quality of life questionnaires were completed by parents nine months after enrolment (QoL<sub>1</sub>) [8, 21] and at two years of age (QoL<sub>2</sub>).

Caregivers of all children signed the informed written consents prior to study enrolment. The study was approved by the Regional Committee for Medical and Health Research Ethics and The Norwegian Data Protection Authority and registered in the Norwegian bio bank registry. The randomised clinical trial part of the study was registered in Clinical Trials.gov, no. NCT00817466 [16].

## Subjects

The mean (range) age of the 358 children in the present study was 5.2 (0.2–13.4) months at enrolment and 24.2 (17.6–34.7) months at the two-year investigation. The children in the bronchiolitis group compared to controls were shorter, more often exposed to second-hand smoke at inclusion and their parents had lower income, lower educational attainment and less often allergic rhinitis or AD (Table 1).

## Methods

Atopic manifestations determined at two years of age, consisted of recurrent bronchial obstruction (rBO) as a proxy for asthma, atopic dermatitis and allergic sensitisation.

**RBO.** RBO was defined as at least three parentally reported episodes of wheeze at two years of age, in line with previous reports [15], with acute bronchiolitis included as one episode.

**Atopic dermatitis.** Atopic dermatitis was defined based upon the modified Hanifin and Rajka's criteria (yes or no) [22], and severity by the SCORing Atopic Dermatitis index (SCORAD) (see Supporting Information for details).

**Allergic sensitisation.** Allergic sensitisation determined by SPT using 17 common inhalant and food allergens with Soluprick SQ allergen extracts, ALK, Hørsholm, Denmark, was defined as positive with at least one mean wheal diameter at least 3 mm greater than the negative control. Further details are given in the Supporting Information.

**Morning salivary cortisol.** Morning salivary cortisol was sampled by the parents on the first morning after enrolment in the bronchiolitis group, otherwise at home and brought to the investigation centre. Two Sorbette hydrocellulose microsponges were applied in the child's mouth as soon as possible after their child's first awakening after 6:00 a.m., before the first meal, and placed in appropriate prepared containers, as described elsewhere [23]. The samples were stored at -86°C and later analysed at Karolinska Institutet, Stockholm, with radioimmunoassay with monoclonal rabbit antibodies Codolet, France).

**The Infant Toddler Quality of Life Questionnaire.** The Infant Toddler Quality of Life Questionnaire (ITQOL-97) [12] completed by the parents included 97 questions within 13 domains scored from 0 (worst) to 100 (best), with no overall score. Accordingly, a change in QoL score is equivalent to the percentage point score change. The Overall health domain consisted of only one item: Is your child's health excellent, very good, good, fair or poor? In line with others [24] and as previously reported [8, 21], with permission from the copyright holder, we recoded the domain Change in health from the original scores from 1–5 to 0–100 (zero meaning worst deterioration of health from one year ago, 50 meaning no change). Four domains (Change in health, General behaviour, Overall behaviour and Getting along) were recorded in children older than 12 months only [8]. The same questionnaire was filled in at



**Table 1. Characteristics and asthma risk factors of the children at two years of age.** All data are given as n and %, unless otherwise stated. The control group is the group from the reference population.

	Bronchiolitis group N = 203	Control group N = 155
Boys n (%)	117 (57.6%)	89 (57.4%)
Age months, mean (SD)	24.2 (3.2)	24.3 (3.7)
Weight kg, mean (SD)	13.2 (1.6)	12.9 (1.5)
Length cm, mean (SD)	87.0 *** (4.1)	88.7(4.2)
Breastfeeding at enrolment n (%)	149 (73.4%)	122 (78.7%)
Second-hand smoke exposure in infancy n (%)	25 (14.4%)**	5 (3.3%)
Second-hand smoke exposure at 2 years	5 (2.5%)	1 (0.7%)
Atopic manifestations defined at 2 years		
At least one n (%)	103 (50.7%)	37 (23.9%)
Two or more n (%)	19 (9.4%)	25 (7.3%)
rBO (at least 3 wheeze episodes) n (%)	98 (48.3%)***	22 (14.2%)
Atopic dermatitis at 2 years n (%)	30 (14.8%)	25 (16.1%)
Allergic sensitisation <sup>1</sup> n (%)	17 (8.4%)	11 (7.4%)
At least one parent asthma n (%)	35 (22.2%)	46 (29.7%)
At least one parent AD n (%)	33 (18.2%)*	46 (29.7%)
At least one parent allergic rhinoconjunctivitis n (%)	62 (34.4%)***	84 (54.2%)
Higher education mothers <sup>2</sup> n (%)	122 (70.1%)***	142 (91.6%)
Higher education fathers <sup>2</sup> n (%)	100 (57.8%)***	129 (83.8%)
Income mothers <sup>3</sup> , mean (SD)	1.92 **	2.13
Income fathers <sup>3</sup> , mean (SD)	2.32 ***	2.59
Caucasian mother n (%)	189 (93.6%)	147 (94.8%)
Caucasian father n (%)	191 (95.0%)	143 (92.3%)

<sup>1</sup>Allergic sensitisation was defined by at least one positive skin prick test to common inhalant and food allergens

<sup>2</sup>Higher education at least three years after secondary school

<sup>3</sup>Annual income before tax. 1: <300.000 NOK. 2: 300.000–500.000 NOK. 3: >500.000 NOK.

\* p<0.05

\*\* p<0.01

\*\*\*<0.001

<https://doi.org/10.1371/journal.pone.0214040.t001>

both time points. For detailed information, see the attachment: ITQOL Survey Profile, provided by the trademark holder. There are 10 infant/ toddler focused concepts or domains. Behaviour is divided into two separately scored domains; Overall behaviour (1 item) and General behaviour (15 items). As there is no overall score, ITQOL-97 can be regarded as several surveys at a time, including three parent-focused items.

**Outcomes and explanatory variables.** The main outcome for our primary aim, QoL<sub>2</sub>, was reported by quantitative values per domain, and secondarily by the number of domains with significantly reduced QoL<sub>2</sub> scores.

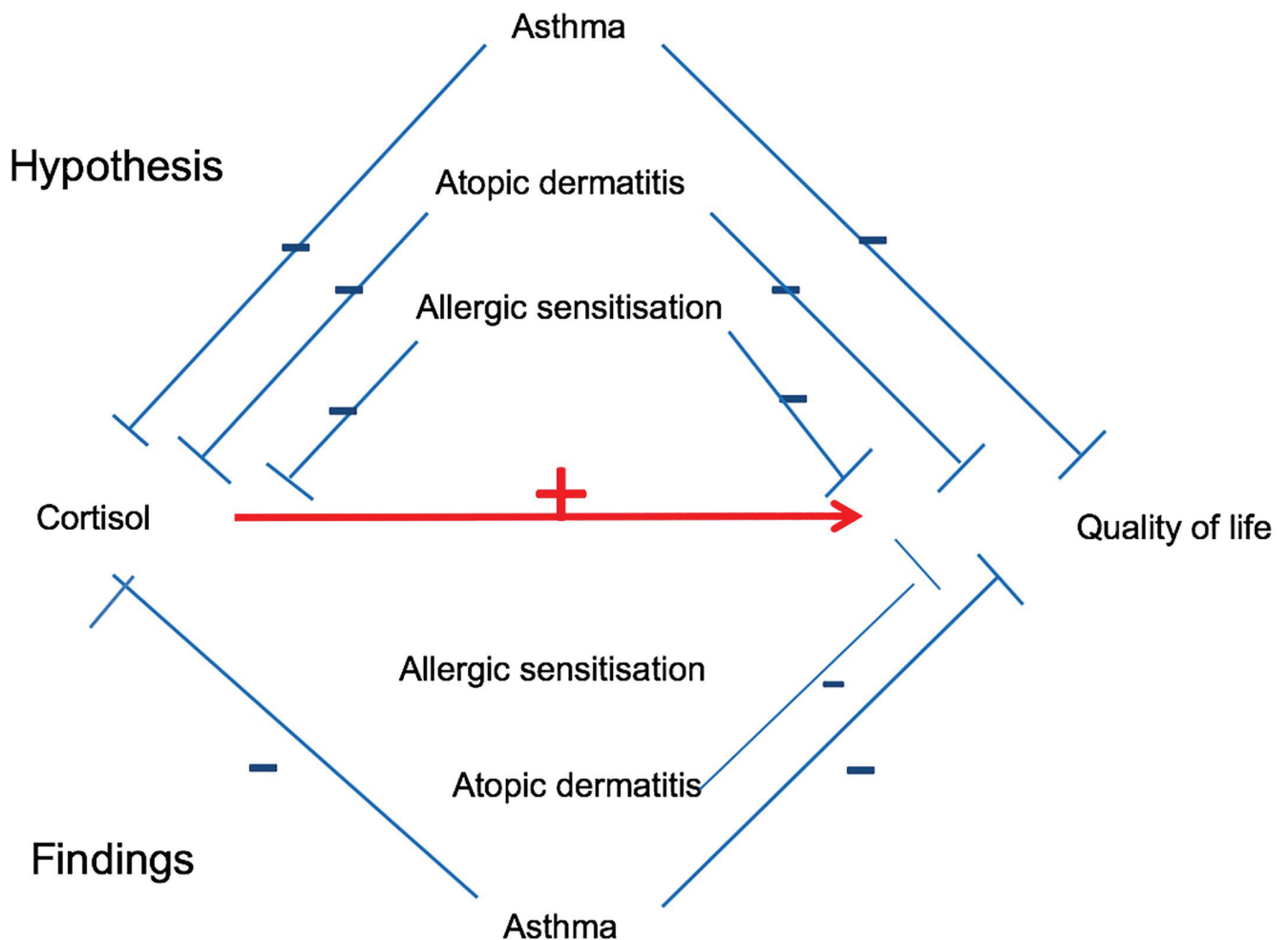
The main explanatory variables for the primary aim were morning salivary cortisol, and the three atopic manifestations rBO, AD and allergic sensitisation at two years of age. Further analyses reported in Supporting Information substituted the respective atopic manifestations by quantitative measures, i.e. the total number of wheeze episodes, the AD severity score SCORAD and the sum of SPT wheal diameters for influence on the associations between morning salivary cortisol and QoL<sub>2</sub>.

The main outcome of the secondary aim was morning salivary cortisol, with QoL<sub>1</sub> as the explanatory factor.

**Statistical analysis**

The bronchiolitis and control groups were compared by Pearson’s chi-square tests for categorical data and Student’s T- test for normally distributed numerical data, and otherwise with Welch test, unless otherwise stated.

Due to non-normality of results and residuals, we used linear robust regression by Huber’s M method [25], for analyses including QoL and cortisol. Each atopic manifestation was included in robust regression models to assess their potential influence on both cortisol and QoL<sub>2</sub>, as well as the associations between the two (see Fig 2, hypothesis). To estimate the relative influence by rBO, AD and allergic sensitisation on QoL<sub>2</sub>, we calculated the percent change of the difference in score for each QoL domain, given per nmol/L change in cortisol. For comparison, we calculated the difference in each QoL domain score that was attributed to a difference in salivary cortisol level of 95<sup>th</sup> versus 5<sup>th</sup> percentile (QoL score at the salivary cortisol level of 95<sup>th</sup> percentile minus QoL score at the 5<sup>th</sup> percentile). Salivary cortisol was studied as a continuous variable, and presented graphically by quartiles.



**Fig 2. Directed acyclic graph showing hypothesised influence on cortisol and QoL<sub>2</sub> by allergic diseases above the red line, and observed influence in the bronchiolitis group below the red line. The red line indicates the net result from the influence of allergic disease on the association between morning salivary cortisol and QoL<sub>2</sub>.**

<https://doi.org/10.1371/journal.pone.0214040.g002>

For graphical presentations of QoL versus cortisol levels and cortisol levels versus atopic manifestations we used data unadjusted for age and gender. In line with previously demonstrated associations between morning salivary cortisol and age as well as gender [23], we decided a priori to analyse age and gender adjusted associations between cortisol and QoL as well between QoL and atopic manifestations. The atopic manifestations were not considered to be possible confounders, as they could be causally associated with both cortisol and QoL<sub>2</sub>.

Using QoL<sub>2</sub> as dependent variable in two-way regression analyses, we tested for interactions between the group affiliation (bronchiolitis or controls) and cortisol, as well as between atopic manifestations and cortisol. Due to interactions between group affiliation and salivary cortisol as well as AD, analyses were stratified by group affiliation.

Possible confounding was assessed by robust regression and considered relevant if the outcome of the model was changed by at least 25% [26] by any of the possible confounders (socioeconomic factors, parental allergic disease, secondary smoke). Confounding by socioeconomic factors was tested by including these factors in multiple regression models, and eliminating the factors with highest p-values stepwise by Hosmer's procedure [26] until only factors with p-values < 0.05 remained, retaining age and gender.

The level of statistical significance was set to  $p < 0.05$  for all analyses.

Analyses were performed with the IBM SPSS Statistics 21 (IBM Corporation, Armonk, New York, USA), and the NumberCruncher Statistical System (NCSS Kaysville, Utah, USA), version 11.

## Results

Children in the bronchiolitis group were significantly more often affected by at least one atopic manifestation at two years of age and had more often rBO than the controls, while AD was similar in the two groups (Table 1).

### Quality of life, salivary cortisol and atopic manifestations at two years of age

The QoL<sub>2</sub> scores varied from 0–100 in five domains, with the smallest score range seen in the domain Getting along (53.3), as shown in Table 2. The bronchiolitis group had a larger reported improvement in health, Change in health, compared with one year ago, while controls scored significantly higher for Overall health and General health (Table 2).

Eight QoL<sub>2</sub> domains were significantly reduced with decreasing salivary cortisol levels ( $p = 0.0001$ – $p = 0.035$ ) among the bronchiolitis group, see Table 3 and Fig 3. In the same group, the association between Overall health and salivary cortisol was significant in boys only, ( $p < 0.0001$ ).

In the controls, General health only was significantly associated and was lower with lower cortisol. The significant decrease of 0.1 percentage point per nmol/L in cortisol level (95% CI 0.0, 0.2,  $p = 0.046$ ) corresponded to a QoL<sub>2</sub> difference of five percent points between children having cortisol levels at the 5<sup>th</sup> vs 95<sup>th</sup> percentile (a difference of 51.6 nmol/L of salivary cortisol). No further analyses were performed in this group, with only one QoL domain significantly associated with salivary cortisol.

The hypothesised (top) and observed (bottom) influence of atopic manifestations on cortisol and QoL<sub>2</sub> are shown schematically in Fig 2. The strongest influence on the associations between cortisol and QoL<sub>2</sub> was exerted by rBO, reducing the associations with 1.4 to 26.9 per cent, followed by changes related to AD ranging from -5.5 to 5.1 and less than 3 per cent changes by allergic sensitisation (Table 2), with all associations between QoL and cortisol remaining significant after including rBO, AD and allergic sensitisation into the regression

**Table 2. Unadjusted weighted means (95% CI) of QoL at two years of age (QoL24m) of children included at hospitalisation for acute bronchiolitis and control children, and descriptive QoL data of all children.**

Domain	Previous bronchiolitis	Control children	All children
	Unadjusted weighted means (95% CI)		Median (min, max)
Overall health	83.4 (81.3, 85.5)**	88.7 (86.3, 91.1)	85.0 (0.0, 100.0)
Physical abilities	100.0 (100.0, 100.0)	100.0 (100.0, 100.0)	100.0 (0.0, 100.0)
Growth and development	94.7 (93.7, 95.6)	95.2 (94.1, 96.3)	97.2 (0.0, 100.0)
Bodily pain/ discomfort	80.5 (78.4, 82.6)	78.5 (76.0, 80.9)	75.0 (8.3, 100.0)
Temperament and moods	84.2 (83.0, 85.4)	83.0 (81.7, 84.4)	84.7 (36.8, 100.0)
General behaviour	84.5 (82.9, 86.0)	85.2 (83.4, 86.9)	85.4 (35.4, 100.0)
Overall behaviour	85.0 (85.0, 85.0)	85.0 (85.0, 85.0)	85.0 (30.0, 100.0)
Getting along	78.8 (77.6, 80.0)	78.5 (77.2, 79.9)	78.3 (45.0, 98.2)
General health	67.1 (65.0, 69.2)****	78.3 (75.9, 80.7)	75.0 (18.2, 100.0)
Change in health	65.2 (62.8, 67.8)****	56.9 (54.1, 59.7)	50.0 (0.0, 100.0)
Parental impact—emotions	91.3 (90.1, 92.6)	91.3 (89.9, 92.7)	92.9 (35.7, 100.0)
Parental impact—time	95.2 (94.2, 96.1)	94.2 (93.1, 95.3)	95.2 (28.6, 100.0)
Family cohesion	79.6 (77.3, 82.0)	80.8 (78.1, 83.5)	85.0 (0.0, 100.0)

\*\* p<0.01

\*\*\*\*p<0.0001

<https://doi.org/10.1371/journal.pone.0214040.t002>

**Table 3. The potential influence of recurrent bronchial obstruction (rBO), atopic dermatitis (AD) and allergic sensitisation (AS) on the associations between Quality of Life (QoL<sub>2</sub>) and salivary cortisol at two years of age is shown for 203 children who had moderate to severe acute bronchiolitis in infancy. The influence by including each atopic manifestation (rBO, AD and AS) is shown as the percentage change of QoL per 1 nmol/L change in salivary cortisol, adjusted for age and gender. Each column includes all children with the observed atopic manifestation, and they are not mutually exclusive.**

Domain (Mean domain score difference <sup>1</sup> by difference between 95 <sup>th</sup> and 5 <sup>th</sup> percentile of cortisol, 51.6 nmol/L)	Change in QoL <sub>2</sub> score per nmol/L unit salivary cortisol	rBO	AD	Allergic sensitisation
		% change in association		
Overall health <sup>2</sup> boys (16.0)	0.31 (0.17, 0.45)****	-20.7	-2.1	-0.5
Overall health girls (-0.0)	-0.00 (-0.16, 0.16)			
Growth and development (3.8)	0.07 (0.02, 0.13)**	-1.4	1.7	0.6
Bodily pain/ discomfort (6.2)	0.12 (0.01, 0.23)*	-8.3	5.4	-2.3
Temperament and moods (6.1)	0.12 (0.06, 0.18)***	-6.9	1.4	-1.5
General behaviour (4.6)	0.09 (0.01, 0.17)*	-6.7	-1.5	1.3
Getting along (4.0)	0.08 (0.02, 0.13)**	-3.0	-0.3	0.9
General health (5.6)	0.11 (-0.00, 0.22) <sup>3</sup>	-26.9	0.4	0.3
Parental impact—Emotions (4.5)	0.09 (0.3, 0.15)**	-6.1	-5.1	1.2
Parental impact—Time (3.0)	0.06 (0.01, 0.10)*	-7.7	-0.1	0.9

<sup>1</sup>QoL score difference equals percentage point difference.

<sup>2</sup>Overall health was gender stratified due to interaction.

\*p<0.05

\*\*p<0.01

\*\*\* p<0.001

\*\*\*\*p<0.0001

<sup>3</sup>p = 0.0517

<https://doi.org/10.1371/journal.pone.0214040.t003>

QoL scores of quartiles of morning salivary cortisol, unadjusted

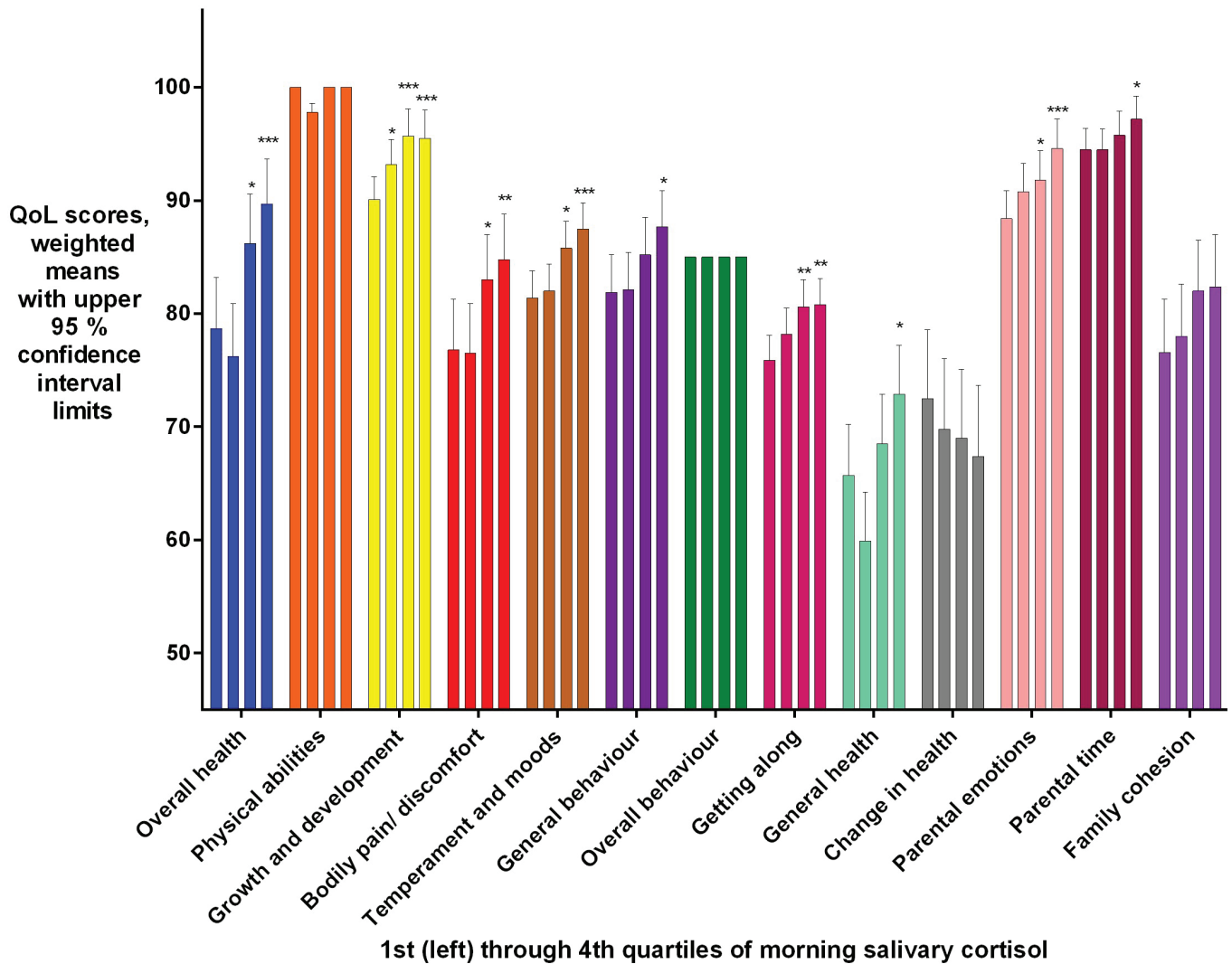


Fig 3. Bronchiolitis group: QoL<sub>2</sub> scores for each domain, unadjusted, for each quartile of morning salivary cortisol, 1<sup>st</sup> quartile lowest cortisol, 4<sup>th</sup> quartile highest. Due to interaction between gender and cortisol for the Overall health domain, this domain was analysed separately for the genders. An association was found only for boys for this domain. For Overall health, results for boys are shown. For the other domains, results for both genders analysed together are shown.

<https://doi.org/10.1371/journal.pone.0214040.g003>

analyses (results not shown). To illustrate the combined estimated effect from rBO and cortisol levels on the Overall health domain, a 24-months-old boy with rBO and low salivary cortisol, at the 5<sup>th</sup> percentile, would have an estimated 23.1 percentage point lower QoL than a boy without rBO who had a high salivary cortisol level, at the 95<sup>th</sup> percentile.

**Table 4. Bronchiolitis group: Change of associations between salivary morning cortisol at and QoL<sub>2</sub> at two years of age by socioeconomic factors, including age, gender, and the following socioeconomic factors: Mother’s education, father’s education, mother’s income, father’s income, ethnicity of father and of mother (Caucasian or not) and secondhand smoke exposure at two years of age.** The socioeconomic factors have been eliminated by Hosmer’s stepdown procedure, finally retaining factors with  $p < 0.05$ . Age and gender have been retained in the models.

Adjusted for/domain	Change of QoL score per nmol/L changed salivary cortisol after adjustment	% influence on change of QoL score by adjustment	Socioeconomic factors retained in the model
Overall Health	0.15 (0.05, 0.26)**	-16.7%	Caucasian father <sup>1****</sup>
Growth and Development	0.07 (0.02, 0.13)*	-3.7%	Caucasian father <sup>1*</sup>
Bodily Pain/Discomfort	0.12 (0.01, 0.23)*		All factors insignificant; eliminated from model
Temperament and Moods	0.11 (0.05, 0.17)***	-8.1%	Caucasian mother <sup>1**</sup>
General Behaviour	0.08 (0.00, 0.16)*	-10.9%	Caucasian mother <sup>1**</sup>
Getting Along	0.06 (0.01, 0.12)*	-18.8%	Education mother <sup>1**</sup> , education father <sup>2**</sup> , Caucasian mother <sup>1**</sup>
Parental Impact—Emotions	0.08 (0.02, 0.14)*	-11.7%	Income father <sup>1*</sup> , Caucasian mother <sup>1****</sup>
Parental Impact—Time	0.05 (0.01, 0.10)*	-13.1%	Caucasian mother <sup>1*</sup> , Caucasian father <sup>1**</sup>

<sup>1</sup> positively associated with QoL domain

<sup>2</sup> negatively associated with QoL domain

\* $p < 0.05$

\*\* $p < 0.01$

\*\*\* $p < 0.001$

\*\*\*\* $p < 0.0001$

<https://doi.org/10.1371/journal.pone.0214040.t004>

We found no significant confounding effect of socioeconomic factors, parental ethnicity and second-hand smoke at two years of age, and these were consequently not included in the final multivariate analyses (Table 4).

The age and gender adjusted salivary cortisol levels at two years were similar in the bronchiolitis group and controls, with a non-significant weighted mean difference of -0.70 (95% CI -3.7, 2.3) nmol/L.

Salivary cortisol was significantly lower among the 120 children with rBO compared to the 238 children without rBO (weighted mean difference -4.1 (95%CI -7.3,-1.0) nmol/L), as shown schematically in Fig 2, and in unadjusted analysis in Fig 4. Neither AD nor allergic sensitisation was significantly associated with morning salivary cortisol at two years of age (Figs 2 and 4).

The QoL<sub>2</sub> was significantly associated with rBO and AD in the bronchiolitis group and with rBO as well as allergic sensitisation in the controls, as reported in Table 5 by percentage point changes in QoL<sub>2</sub> scores by the atopic diseases.

### QoL<sub>1</sub> and salivary cortisol at two years

Lower morning salivary cortisol at two years of age was significantly associated with lower QoL nine months after enrolment within the bronchiolitis group by age and gender adjustment, as shown in Table 6. No significant associations were observed between morning salivary cortisol and QoL<sub>1</sub> among the controls.

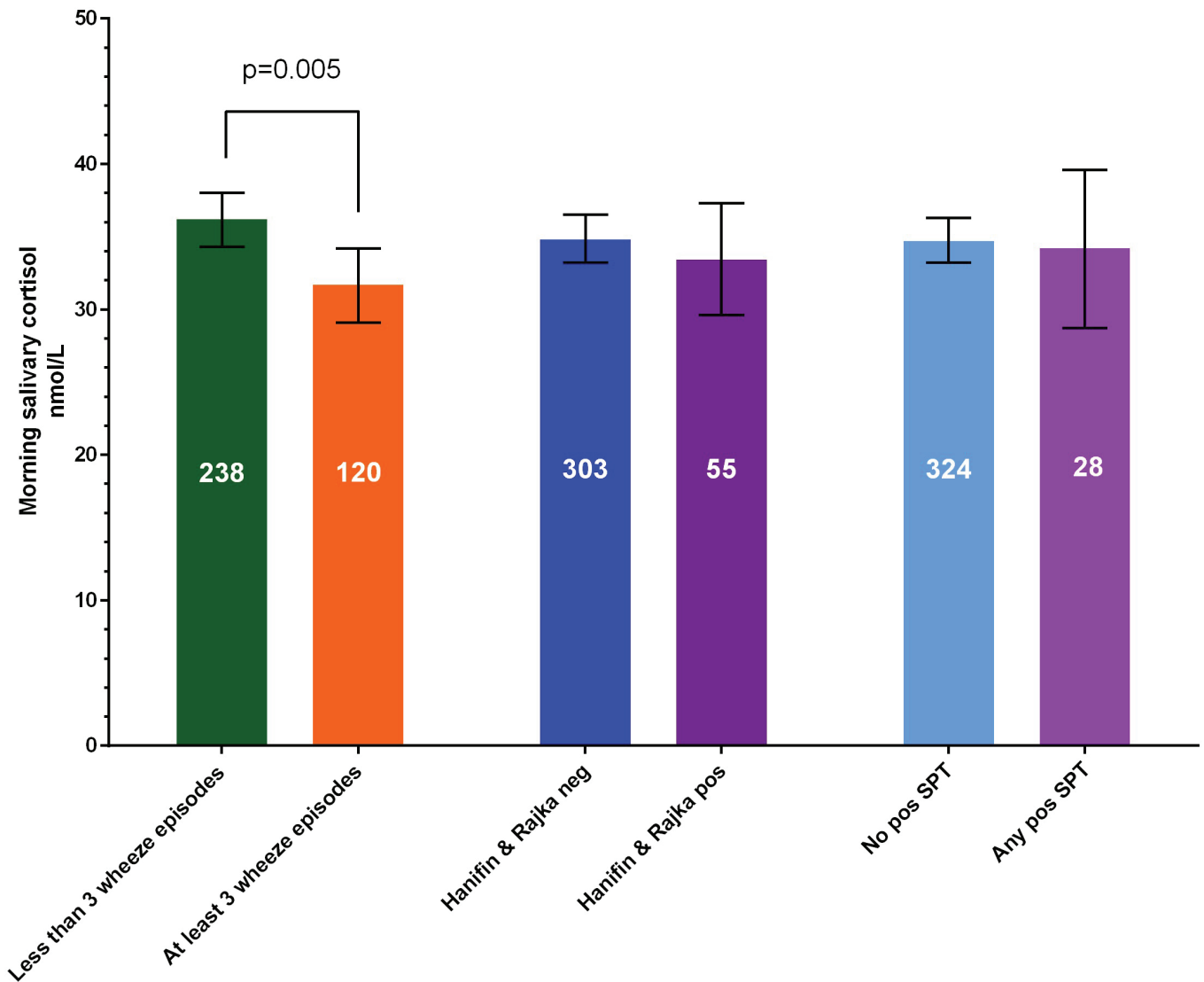


Fig 4. Morning salivary cortisol in children of the bronchiolitis group and controls together, without recurrent bronchial obstruction (rBO), defined as at least three wheeze episodes, compared to with rBO, no AD vs. with AD as well as with no or any positive skin prick test (SPT) to common inhalant and food allergens.

<https://doi.org/10.1371/journal.pone.0214040.g004>

## Discussion

By this explorative add-on to the Bronchiolitis All SE-Norway study, including children enrolled at hospitalisation for acute bronchiolitis and control children, we confirmed the hypothesis that *low* cortisol levels at visits not requiring acute hospitalisation may contribute to development of asthma in children with moderate to severe acute bronchiolitis in infancy, but not in controls. The observed reduction in most QoL domains with lower cortisol levels was partly explained by rBO, whereas the impact of atopic dermatitis was less clear. No influence was observed by allergic sensitisation. Furthermore, we could confirm our second hypothesis, that reduced QoL some months after hospitalisation for moderate to severe bronchiolitis may be a marker of chronic stress, by demonstrating that lower QoL at a mean age of

**Table 5. The impact of allergic diseases on QoL<sub>2</sub> is given for each domain as mean difference from not having the condition, given as percentage points (95% CI), adjusted for age and gender.** Results are stratified for enrolment group based upon interaction analyses. As an example; the negative association of General health (GH) with rBO is stronger in the bronchiolitis group than among controls, both being statistically significant.

	Recurrent bronchial obstruction		Atopic dermatitis		Allergic sensitisation	
	bronchiolitis group	controls	bronchiolitis group	controls	bronchiolitis group	controls
<b>OH</b>	-12.3 (-16.5, -8.2)****	-6.6 (-13.9, 0.7)	-5.5 (-11.7, 0.6)	-6.3 (-13.6, 0.9)	-0.7 (-8.6, 7.2)	-11.7 (-21.6, -1.9)*
<b>PA</b>	-1.2 (-2.1, -0.4)**	Not done	-0.0 (-0.0, 0.0)	-2.1 (-3.2, -1.0)***	0.0 (-0.2, 0.2)	Not done
<b>GD</b>	-3.5 (-5.8, -1.2)**	-1.3 (-4.3, 1.8)	-2.5 (-5.8, 0.7)	-1.2 (-4.2, 1.8)	-0.1 (-4.3, 4.0)	-1.9 (-6.4, 2.6)
<b>BD</b>	-6.5 (-10.8, -2.1)**	-7.3 (-14.7, 0.1)	-7.2 (-13.4, -0.9)*	-1.7 (-8.6, 5.3)	4.2 (-3.6, 12.1)	-6.4 (-16.8, 4.0)
<b>TM</b>	-3.1 (-5.5, -0.7)*	-4.1 (-7.8, -0.5)*	-5.1 (-8.4, -1.8)**	1.0 (-2.6, 4.5)	-2.5 (-1.8, 6.8)	-1.3 (-6.5, 3.9)
<b>GB</b>	-4.7 (-7.9, -1.4)**	-0.7 (-5.5, 4.1)	-5.3 (-9.9, -0.7)*	-4.1 (-8.8, 0.6)	-0.8 (-6.8, 5.2)	-7.0 (-13.7, -0.3)*
<b>OB</b>	-0.0 (-0.0, 0.0)	Not done	0.0 (0.0, 0.0)	Not done	0.0 (-0.0, 0.0)	Not done
<b>GA</b>	-1.9 (-4.2, 0.4)	-4.0 (-7.9, -0.0)*	-6.2 (-9.3, -3.0)***	-0.9 (-4.8, 2.9)	-0.4 (-4.6, 3.8)	-8.0 (-13.6, -2.4)**
<b>GH</b>	-13.8 (-17.8, -9.8)****	-6.8 (-12.9, -0.7)*	-5.8 (-12.0, 0.3)	-7.0 (-13.0, -1.0)*	0.1 (-7.9, 8.1)	-12.2 (-20.5, -3.9)**
<b>CH</b>	6.9 (0.8, 13.0)*	6.8 (-0.6, 14.2)	7.8 (-0.9, 16.4)	3.1 (-3.3, 9.6)	-1.0 (-12.3, 10.3)	7.3 (-2.8, 17.4)
<b>PE</b>	-4.0 (-6.4, -1.5)**	-2.3 (-5.9, 1.4)	-5.7 (-9.2, -2.2)**	-1.6 (-5.2, 2.0)	-0.2 (-4.7, 4.3)	-5.8 (-10.7, -1.0)*
<b>PT</b>	-2.5 (-4.5, -0.5)*	-2.1 (-5.0, 1.0)	-1.9 (-4.8, 1.0)	-1.9 (-4.9, 1.0)	-0.0 (-3.7, 3.7)	-4.3 (-8.3, -0.2)*
<b>FC</b>	-0.1 (-4.7, 4.5)	0.3 (-7.4, 8.0)	-5.4 (-11.9, 1.1)	0.7 (-6.7, 8.1)	-0.0 (-3.7, 3.7)	-1.8 (-12.3, 8.7)

Not done refers to analyses that were not applicable due to strong correlations between some of the included variables.

\*p<0.05

\*\*p<0.01

\*\*\*p<0.001

p<0.0001

OH = Overall health; PA = Physical abilities; GD = Growth and development; BD = Bodily pain/ discomfort; TM = Temperament and moods; GB = General behaviour; OB = Overall behaviour; GA = Getting along; GH = General health; CH = Change in health; PE = Parental impact—emotions; PT = Parental impact—time; FC = Family cohesion

<https://doi.org/10.1371/journal.pone.0214040.t005>

14 months was associated with lower cortisol levels at two years of age. Finally, the associations at two years of age between cortisol and QoL could only partly be explained by rBO.

We are not aware of other studies comparing QoL and morning salivary cortisol in children. We have previously found that infants with acute bronchiolitis have higher morning salivary cortisol than controls [23], indicating acute stress. Others have found other signs of acute stress in acute bronchiolitis with respiratory syncytial virus, differing from other infections

**Table 6. Significant associations between QoL<sub>1</sub> (at a mean age of 14 months and morning salivary cortisol at two years are presented as the mean age and gender adjusted change in cortisol level per change scores per domain.**

	Change in cortisol nmol/L per QoL14m score change
Overall health	0.17 (0.02, 0.32)*
Physical abilities	0.92 (0.41, 1.43)***
Growth and development	0.34 (0.09, 0.60)**
Temperament and moods	0.35 (0.12, 0.59)**
General health	0.17 (0.01, 0.33)*
Parental impact—emotions	0.45 (0.19, 0.71)***
Parental impact—time	0.28 (0.11, 0.46)**

\*p<0.05

\*\*p<0.01

\*\*\*p<0.001

<https://doi.org/10.1371/journal.pone.0214040.t006>



and acute diseases [27]. An explanation of a seeming contradiction, that at inclusion with acute bronchiolitis the cortisol levels were higher, but later lower in subjects with low QoL as signs of chronic stress, can be that these infants, while hospitalised with moderate to severe bronchiolitis, had not yet developed asthma or were not hit by a blunted cortisol response seen in asthmatic children with time, or were so profoundly affected by acute stress that even subjects with a blunted cortisol response managed to raise cortisol. Reduced basal morning cortisol levels observed in children with asthma, also without concurrent use of inhaled corticosteroids (ICS) [3], may on the other hand indicate chronic immunological stress, see Supporting Information. The subsequent blunted cortisol responses to acute stress reported in subjects with asthma related to a disturbance of the hypothalamus-pituitary-adrenal (HPA) axis differs from the chronic stress in non-atopic children that can lead to a higher cortisol response [20, 28]. A possible connection between our finding of lower morning salivary cortisol in children with lower QoL in the bronchiolitis group whereas others found lower cortisol responses to acute stress in children with asthma [20] can be that asthmatic children may not only have a blunted response to acute stress, but also a lower diurnal variation as well as a possibly lower cortisol awakening response, which is influenced by acute stress [29]. In line with our findings, a tendency for decreased cortisol levels has been found in young children with at least three wheeze episodes [30]. A possible explanation why a lower cortisol response to acute stress in children and adults with AD was found by Kojima et al. [31], but not in our nor another study, can be that AD had lasted longer or were more serious than in our or the ALADDIN cohort study [30].

Reduced QoL after acute bronchiolitis [13, 32], may partly be expressions of chronic physical, and psychological stress. Concerns of the parents of the children of the bronchiolitis group, as indicated by the Parental impact—emotions and Parental impact—time domains in the present study, seem to be associated with the children's cortisol levels. The associations between cortisol and QoL in domains reflecting expressions of pain, moods and behaviour, i.e. Bodily pain/ discomfort, Temperament and moods, General behaviour and Getting along, partly influenced by rBO, may also indicate a role of psychological stress in the development of asthma.

The 16 percentage point difference in Overall health in boys with low versus high salivary cortisol is likely to be clinically relevant as they are comparable to the eight percentage point General health differences between children with and without asthma-like symptoms reported from the Generation R study [33].

The observed association between Overall health and morning salivary cortisol at two years of age was significant among both genders analysed together, but only in boys by gender stratified analyses performed for this domain due to interaction. This may be related to our finding of significantly higher salivary cortisol levels in girls compared to boys at two years in the Bronchiolitis all SE-Norway study [23].

The lack of significant associations between allergic sensitisation and QoL in the bronchiolitis group and between allergic sensitisation and salivary cortisol may have several explanations. In our study less than 10 per cent of the subjects were sensitised to at least one allergen, limiting the likelihood of observing significant associations. On the other hand, allergic sensitisation may not affect QoL before allergen exposure causes symptoms, which for inhalant allergens occur more frequently with increasing age [34].

Our finding that reduced QoL about one year of age was associated with lower salivary cortisol at two years of age is consistent with our recently published finding in the same study population that in addition to having been hospitalised for acute bronchiolitis, disease severity and asthma risk factors as well as AD at inclusion were associated with reduced QoL at 14 months of age [8, 21].

The direct clinical implications of our findings remain unclear at present. The maintenance of statistical significance of the influence of cortisol on QoL after adjusting for rBO indicates a role of other factors than an obvious proxy for asthma on the concordant relationship between morning salivary cortisol and QoL after acute bronchiolitis. However, our study suggests that in addition to rBO, also acute moderate to severe infant bronchiolitis, disposing for asthma, may play a role in the association between future salivary cortisol and QoL in subjects who have not yet developed asthma. Although the influence of the asthma proxy rBO dominated the association between cortisol and QoL at two years, the associations were significant also among children in the bronchiolitis group without rBO, see Supporting Information. Together these observations suggest that children who have acute bronchiolitis in infancy and reduced QoL some months later may be vulnerable to chronic stress, observed by lower salivary cortisol and reduced QoL at two years of age. Thus, our study supports a role of chronic stress indicated by lower cortisol levels in development of asthma.

In line with previous studies finding marginally lower cortisol in adolescents with low socioeconomic status [35], we included socioeconomic data as well as second-hand smoking into regression analyses. However, none of these factors were found to be significant confounders, possibly reflecting the overriding effects by atopic diseases in the children, as well as a low frequency of second-hand smoke in our cohort.

### Strengths and limitations

The study strengths include a prospective design of a reasonably large group of children included in infancy with acute bronchiolitis and atopic disease, a control group of similar age recruited from a general population with a frequency of atopic manifestations (rBO, AD and allergic sensitisation) on the same levels as other two year old children in Norway [36], a high retention rate at follow-up investigations, repeated measurements and stringent clinical characterisation of the subjects. Also, the findings appear robust, as the associations remained significant after relevant adjustments.

The lack of significant associations between QoL and salivary cortisol in the control group may be due to the relatively few subjects with rBO, most consistently associated with reduced QoL and salivary cortisol, and that the control children may be more heterogeneous, possibly with a lower risk of future asthma development, or that the control children in general had a higher QoL.

As previously reported [8, 21], we decided a priori not to adjust for multiple analyses, as the QoL domains were not independent from each other. Also, the associations with the different QoL domains point in the same direction, limiting the likelihood of incidental findings. As expected, the Change in health domain improved more among children with previous acute bronchiolitis than among controls, in line with findings in children with chronic diseases [13].

Our use of single morning salivary cortisol measurements may be a limitation in terms of identifying diurnal variation, but improved feasibility of obtaining such measurements. However, previous studies of single morning measurements [3] and the lack of significant day-to-day variation between three samples taken at 4- to 8-day intervals [37], suggest that single measures may reflect habitual morning cortisol. We sampled as soon as possible after the first awakening after 6:00 a.m. [23], possibly encompassing a morning awakening response and the top circadian morning cortisol [38].

### Conclusion

At two years of age most QoL domains decreased with lower morning salivary cortisol among children who had been hospitalised for acute bronchiolitis in infancy, but for one domain only

among controls. Recurrent bronchial obstruction and, to a limited extent atopic dermatitis weakened these associations that nevertheless remained significant. After bronchiolitis, lower QoL in one-year-old children was associated with lower salivary cortisol at two years.

## Supporting information

**S1 Table. Severity of the allergic diseases of the 358 children of the study population.**  
(DOCX)

**S2 Table. Per cent change of the association between QoL24m and morning salivary cortisol at two years of age, by adjusting for the total number of wheeze episodes, SCORAD index and the sum in mm of positive skin prick tests (except for histamine) in addition to age and gender, bronchiolitis group.** Only domains with statistically significant associations are presented.  
(DOCX)

**S3 Table. Associations between QoL24m and cortisol, nmol/L (95% CI), adjusted for age and gender in children without RBO, bronchiolitis group; only domains significantly associated with cortisol when children with RBO are included are shown.**  
(DOCX)

**S1 File.**  
(DOCX)

## Acknowledgments

We warmly acknowledge all participating children and parents and the members of the Bronchiolitis Study Group, and study nurses, see Supporting Information, and the several hundred study staff that were involved in recruiting patients and running the study. Warm thanks also to Johan Alm and Ann-Christine Sjöbeck, Department of Clinical Science and Education, Karolinska Institutet, Stockholm, Sweden, for the analysis of the salivary cortisol samples.

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# Quality of life, salivary cortisol and allergic diseases in young children Supporting Information

## Study design

### Inclusion criteria

Inclusion criteria for the bronchiolitis group were clinical signs of bronchiolitis, as defined by Court: Illness mainly affecting infants, especially in the first 6 months of life. Rapid respiration, dyspnea, wheezing, chest recession, cough, rhonchi and rales are very frequent. Visible distension of the chest and increased pulmonary translucency on the chest radiograph are frequent and of high diagnostic significance. Upper respiratory features, especially nasal discharge and a red pharynx are frequent. Fever is very frequent, but high fever uncommon (1). To be enrolled at hospital admission for acute bronchiolitis, the bronchiolitis had to be rated as moderate to severe, with at least 4 of a maximum of 10 points of a scale published by S. Kristjansson et al. (2), and described by H.O. Skjerven et al. (3), and the age should be below 12 months of age. Inclusion criteria for the children of the general population (4) was age below 12 months.

### Exclusion criteria

For the bronchiolitis group, exclusion criteria were any severe or chronic disease that might significantly influence the progression of acute bronchiolitis, more than one previous episode of obstructive airways disease or more than four weeks with lower airways disease symptoms and, or, use of inhaled corticosteroids in the previous four weeks. Exclusion criteria for the population based control group were any severe underlying disease, for example heart, lung, immunological, neurological or oncological disease.

### Subjects

Significantly more of the subjects from the control group met at the two year of age follow-up examination and yielded QoL answers as well as successful cortisol samples, than of the bronchiolitis group. From the control group, we obtained QoL and cortisol results from 155 children, 64.6 %, and from the bronchiolitis group, from 203 children, 50.2 %. Children without QoL or salivary cortisol data



or who did not meet at two years of age (201 from the original bronchiolitis group and 85 controls) differed from those who met when it came to parental education (mothers 3.87 vs. 4.34,  $p < 0.001$ , fathers 3.87 vs. 4.08,  $p < 0.01$ ), ethnicity (Caucasian mother 89.7 % vs. 94.4 %,  $p < 0.05$ , Caucasian father (88.8 % vs. 94.4 %,  $p < 0.01$ ), and smoking in the homes 15.3 % vs. 9.2 %), but not with respect to parental asthma, parental rhinoconjunctivitis or atopic dermatitis at enrolment.

## Methods

### Recurrent bronchial obstruction (rBO)

At the follow-up visit, the parents were asked about the number of wheezing episodes in their child's first year and second year, to provide a measure of asthma severity for supplementary analyses. At enrolment, the parents had been asked if their child had had more than one episode of wheezing earlier, because this was an exclusion criterion for the bronchiolitis group. The parents of the control subjects were asked about the number of wheeze episodes also at enrolment in infancy. The number of wheezing episodes was adjusted for information at enrolment, in case this was forgotten, and an episode of bronchiolitis was counted as one episode of wheezing.

### Hanifin and Rajka criteria for AD

We defined fulfilment of the criteria this way: 1. At least three of the four main criteria: pruritus; dermatitis in the face and/or the extensor surfaces of the extremities; chronic or relapsing dermatitis; personal or family history of atopy (asthma, allergic rhinitis, AD). 2. In addition, at least three minor features: Onset in childhood; deterioration by emotional factors; deterioration by environmental factors; products may irritate the skin, e.g. wool, soap; tendency toward cutaneous infections with virus and bacteria; facial pallor or facial erythema; hypopigmented spots; dark skin below the palpebral fissures; Morgan infraorbital fold; angular cheilitis; anterior neck folds; ichthyosis; palmar hyperlinearity; keratosis pilaris; dermatitis on hands and feet ("winter feet"); white dermographism; perifollicular accentuation; nipple eczema; recurrent conjunctivitis; dry skin.

Compared to the original Hanifin and Rajka criteria; five of the original 23 minor features were not asked or examined for: The two ophthalmological criteria originally included by Hanifin and Rajka,

keratoconus and anterior or subcapsular cataracts, were not included and would not have been likely to have given any positive answers. Some of the side criteria were grouped together as one criterion in the original definition. Furthermore, food intolerance, elevated serum IgE or type I skin reactivity were not included, but type I skin reactivity was included as an own atopic manifestation not included in the definition, as described in the main text.

## **Severity assessment of atopic dermatitis**

Severity was rated by the SCORing AD (SCORAD) index (5), in all children with signs of AD. The higher of the two values, obtained by a trained physician and a trained nurse was used for analyses. In unrated children, the indexes were set to zero.

## **Allergic sensitisation**

Allergic sensitisation was determined by skin prick test (SPT) was tested with the following allergens: dog dander, cat dander, house dust mite (*Dermatophagoides pteronyssinus*), birch, timothy and mugwort pollen, the mould species *Cladosporium herbarum* and *Alternaria tenuis*, hen's egg white, peanut, almond, hazelnut, wheat, cow's milk, soy, cod, shrimp and positive and negative controls (Soluprick, Soluprick SQ, ALK, Hørsholm, Denmark). The sum of allergen wheal diameters exceeding negative control (6) were included in disease severity analyses,

## **Results**

Median (min.-max.) duration from this first QoL (QoL<sub>1</sub>) survey to the follow-up clinical visit and second QoL survey (QoL<sub>2</sub>) with the same questionnaire was 9.6 (5.4-13.8) months.

RBO was negatively associated with nine QoL<sub>2</sub> domains of the bronchiolitis group and three domains of the control group, and positively with one domain of the bronchiolitis group, Change in health (compared to one year ago), AD negatively with QoL<sub>2</sub> in seven domains in the bronchiolitis group and in one domain in the control children, whereas allergic sensitisation was not significantly associated with any QoL<sub>2</sub> domain in the bronchiolitis group, but negatively with six domains in the control group.



S1 Table indicates the severity of the allergic diseases and S2 Table show results from adjusting for the continuous expressions of the atopic manifestations. In the bronchiolitis group, adjusting for the number of wheeze episodes, reduced the associations between cortisol and QoL further for seven domains. Statistical significance disappears for Bodily pain/ discomfort and General behaviour, illustrating the impact by wheeze on associations between these QoL<sub>2</sub> domains and cortisol. Adjustment for the severity of AD or degree of allergic sensitisation did not turn any associations to insignificance. Also in children without rBO, we found an association in the bronchiolitis group between cortisol and QoL<sub>2</sub> in the domains Overall health (boys), Growth and development, Temperament and moods, General behaviour, Parental impact – emotions and Parental impact – time. Details are shown in S3 Table.

## Immunological aspects

The severity of acute bronchiolitis has been associated with plasma cortisol suppressing the T-helper cell type 1 (Th1) immune response (7), possibly leading to a shift to a Th2 response in acute bronchiolitis through inhibition of the interferon gamma response acting directly on T cells or indirectly through IL-12. Glucocorticoids may stimulate the secretion of IL-4 and IL-10, enhancing the Th-2 response, and stimulate Th2-cells directly (8), as well as possibly suppressing Th2-inflammation (9).

## The Bronchiolitis Study Group

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## S1 Table

### Severity of the allergic diseases of the 358 children of the study population

	Bronchiolitis group <sup>1</sup> N=206	Control group N=152
SCORAD <sup>1</sup> , mean, SD	7.0 (9.5)	7.8 (13.7)
SCORAD 0 n (%)	102 (49.5%)	97 (63.8%)
Mild AD (SCORAD<25) n (%)	91 (44.2%)**	40 (26.3%)
Moderate AD (SCORAD 25-50) n (%)	12 (5.8%)	10 (6.6%)
Severe AD (SCORAD>50) n (%)	1 (0.5%)	5 (3.3%)
Total no. of wheeze episodes, mean (SD)	5.7 (8.4)***	1.1 (2.1)
Sum SPT <sup>2</sup> , mean, SD	1.3 (3.3)	1.5 (4.2)

<sup>1</sup>SCORAD rating took place when signs of AD were seen. No SCORAD data were set to zero.

<sup>2</sup>Sum of wheal diameters, exceeding negative controls, histamine controls not included

\* p<0.05 \*\* p<0.01 \*\*\*<0.001

**S2 Table**

Per cent change of the association between QoL24m and morning salivary cortisol at two years of age, by adjusting for the total number of wheeze episodes, SCORAD index and the sum in mm of positive skin prick tests (except for histamine) in addition to age and gender, bronchiolitis group. Only domains with statistically significant associations are presented.

	Change by adjustment for no. of wheeze episodes	Change by adjustment for SCORAD	Change by adjustment for the sum of positive SPT wheal diameters	Change by adjustment for no. of wheeze episodes, SCORAD and the sum of positive SPT wheal diameters
<b>Overall health</b>	-22.8 %	-4.6 %	0.5 %	-25.1 %
<b>Growth and development</b>	-16.6 %	1.2 %	0.4%	-13.0 %
<b>Bodily pain/ discomfort</b>	-25.9 % <sup>1</sup>	3.1 %	-1.1 %	-21.4 % <sup>1</sup>
<b>Temperament and moods</b>	-15.7 %	0.8 %	0.0 %	-12.9 %
<b>General behaviour</b>	-13.2 % <sup>1</sup>	-0.5 %	-0.6 %	-13.2 % <sup>1</sup>
<b>Getting along</b>	-8.1 %	3.4 %	0.4 %	-7.3 %
<b>Parental impact - emotions</b>	-17.5 %	3.1 %	-1.1 %	-14.7 %
<b>Parental impact - time</b>	-16.7 %	-0.5 %	1.3 %	-11.9 %

<sup>1</sup>No longer statistically significant after adjustment

### S3 Table

**Associations between QoL24m and cortisol, nmol/L (95% CI), adjusted for age and gender in children without RBO, bronchiolitis group; only domains significantly associated with cortisol when children with RBO are included are shown.**

Domain	n	Change QoL score per nmol/L cortisol
<b>Overall health</b>	95	0.14 (-0.01, 0.28)
<b>Overall health boys<sup>1</sup></b>	50	0.31 (0.13, 0.50)**
<b>Overall health girls</b>	45	0.01 (-0.21, 0.23)
<b>Growth and development</b>	103	0.07 (0.01, 0.14)*
<b>Bodily pain/ development</b>	104	0.14 (-0.01, 0.28)
<b>Temperament and moods</b>	105	0.10 (0.02, 0.18)*
<b>General behaviour</b>	105	0.10 (-0.00, 0.21)
<b>Getting along</b>	103	0.06 (-0.02, 0.14)
<b>Parental impact – emotions</b>	104	0.09 (0.01, 0.16)*
<b>Parental time</b>	102	0.07 (0.01, 0.12)*

<sup>1</sup>Stratified for genders due to interaction

\* p<0.05    \*\* p<0.01



## ITQOL: Infant Toddler Quality of Life Questionnaire (For content review only)

The Infant Toddler Quality of Life Questionnaire™ (ITQOL) was developed for use in infants and toddlers at least 2 months of age up to 5 years. The Infant Toddler Quality of Life Questionnaire™ (ITQOL) adopts the World Health Organization's definition of health, as a state of complete physical, mental and social well being and not merely the absence of disease. The survey was developed following a thorough review of the infant health literature and a review of developmental guidelines used by pediatricians. The 47 item short-form and the 97 item full-length versions measure the same concepts; just with fewer items. For each concept, item responses are scored, summed, and transformed to a scale from 0 (worst health) to 100 (best health). Summary scoring and norms are not yet available for either length of the ITQOL.

Completion times can vary depending on a complex host of issues such as the setting, context, age, cognitive functioning, language, layout, etc. Time frames for response options vary - for example some scales ask about the past 4 weeks, the global health items asks about health "in general" and the global change items asks as compared to one year ago. There are some skip patterns as the Behavior Scales and Change in Health items are not appropriate for infants less than 12 months of age. Response options for both lengths of the ITQOL scales are five levels, with the exception of Parent-Time Limitations which is 4 levels. The item stems and statements (i.e. survey content) provided below give you an indication of the different response options (e.g. How much, how often etc.) used throughout the surveys.

### Infant Toddler Quality of Life Questionnaire – 47 Items

Infant/toddler focused concepts:

- How would you rate your child's health?
- Considering your child's age and abilities, has he/she been limited in any of the following because of health or learning problems? Feeding/nursing/eating; Sleeping; Grasping; Rolling over; Playing; Taking steps or walking
- How satisfied are you with your child's: Physical growth and development? Motor development? Responsiveness to others? Language development?
- How much bodily pain or discomfort (due to gas, teething, injury, illness) has your child had anywhere in his/her body?
- How often has your child had discomfort or pain anywhere in his/her body?
- How much of the time did your child seem: Less active than usual? Bothered or upset?; "Just not him/herself"?; Cheerful?; Easily upset?; Alert?
- How much do you agree/disagree with each statement for your child? My child's behavior is sometimes difficult to manage; My child seems to misbehave more often than other children I know; People have complimented me on my child's behavior. Others have complained about my child's behavior.
- Compared to children of the same age, how would you rate your child's behavior overall?
- How often did your child: Have behavior that was difficult to manage? Get along with other children? Throw tantrums? Respond positively to affection? Act withdrawn? Act his/her age? Listen to or follow directions?
- How true or false is each statement for your child? My child seems to be less healthy than other children I know; My child has never been seriously ill; When there is something going around my child usually catches it; I expect my child will have a very healthy life; I worry about my child's health more than other people worry about their children's health.
- Compared to one year ago, how would you rate your child's health now?

Parent-focused concepts:

- How much anxiety or worry did each of the following cause you? Your child's physical health; emotional well-being/behavior/temperament; learning abilities or cognitive development; ability to interact with others
- Were you LIMITED in the amount of time YOU had for your own personal needs due to problems with your child's: physical health; emotional well-being/behavior/temperament; learning abilities or cognitive development; ability to interact with others
- How would you rate your family's ability to get along with one another?

**Infant Toddler Quality of Life Questionnaire – 97 Items**

Infant/toddler focused concepts:

- Overall health (1 item)
- Amount of limitation in physical activities such as eating, sleeping, grasping and playing due to health or learning problems (10 items)
- Satisfaction with development (physical growth, motor, language, cognitive), habits (eating, feeding, sleeping) and overall temperament (10 items)
- Amount, frequency of bodily pain/discomfort and the extent to which pain/discomfort interferes with normal activities (3 items)
- Frequency of certain moods and temperaments, such as sleeping/eating difficulties, crankiness, fussiness, unresponsiveness, playfulness and alertness (18 items)
- Perceptions of current, past and future behavior (12 items)
- Overall behavior (1 item) and frequency of behavior problems, such as following directions, hitting, biting others, throwing tantrums, and easily distracted. Frequency of positive behaviors, such as ability to cooperate, appears to be sorry, and adjusts to new situations (15 items)
- Perceptions of current, past and future health (11 items)
- Perceptions of changes in health over the past year (1 item)

Parent-focused concepts:

- Amount of worry experienced by parent due to child's eating/sleeping habits, physical and emotional well-being, learning abilities, temperament, behavior and ability to interact with others in an age-appropriate manner (7 items)
- Amount of time limitations experienced by parent (time for his/her own needs) due to child's eating/sleeping habits, physical and emotional well-being, learning abilities, temperament, behavior and ability to interact with others in an age-appropriate manner (7 items)
- Rating of family's ability to get along with one another (1 item)