AIRWAY INFLAMMATION IN SCHOOL-AGED CHILDREN WITH ASTHMA

NGUYEN THI DIEU THUY

MD

A Thesis Submitted for the Degree of

Doctor of Philosophy

August 2007

Faculty of Health

School of Medicine and Public Health

University of Newcastle
STATEMENT OF ORIGINALITY

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

ACKNOWLEDGEMENT OF AUTHORSHIP/COLLABORATION

I hereby certify that the work embodied in this Thesis is the result of original research, the greater part of which was completed subsequent to admission to candidature for the degree (except in cases where the Committee has granted approval for credit to be granted from previous candidature at another institution).

Signature: ...............................................Date...............................................
ACKNOWLEDGMENTS

Four years living in Australia, it was one of the special periods in my life. Over the past four years, I have learnt not only about the process involved in research, particularly asthma, the area I am very interested, but I have also learnt about Australian people and Australian culture. Any time and any where, I always received the unconditional support from all of the fantastic people in my study group.

I would like to thank and acknowledge especially my supervisors, Professor Peter Gibson and Professor Michael Hensley for accepting me when you did not know who I am. Working with me, you both accepted the challenge, the difficulty and unexpected troubles. I am very thankful for your patience and encouragement throughout the duration of my study. Peter, the knowledge, methods and the techniques you trained me are invaluable, they will stick with me in my future research. Michael, thank you for your support me during my research and the time I am living in Australia. I am very grateful to A/Prof Bruce Whitehead for his assistance in the clinic as well as his interest and advice in my study.

My study could not have been completed without clinical assistance of Ms Noreen Bell. Recruitment and working with children are very hard, and I am highly appreciative of your clinical support.
I also gratefully acknowledge all of the laboratory staff: Ms Naomi Fibbens, Ms Rebecca Oldham, Ms Kelly Fakes, Ms Michelle Gleeson, and Joanna Mimica, thank you for your co-operation and support during my projects.

I would like to acknowledge Dr Jodie Simpson, who has shared her knowledge and the experience in the research with me. I also wish to thank Dr Lisa Wood. She has organized and supported with studies of the oxidative stress markers in children with asthma. I appreciate the statistical assistance of Ms Heather Powell. My study could not have been completed without the support of all staff of the Airway Research Centre and their children who are volunteers as healthy controls in my projects. Thank you so much Mr Terry Grissell, Ms Nicole Ryan, Ms Philippa Talbot, Ms Vanessa McDonald, Ms Lisa Wood, Ms Heather Powell and all their children. I also would like to thank all staff in my study group, Ms Deborah Hall, Ms Vanessa Murphy, Ms Katie Baines, Ms Glenda Walker, Ms Joanne Smart; you are very friendly and have supported me during my study.

A special thank you to Ms Helen Bryce, an International officer, for being a friend and helpful at the time I am studying in Australia.

Finally, I want to especially thank my big family, my father, my mother, my husband, my brother and my daughter, for always unconditional support throughout many years of my study. You have given me the opportunities that have allowed me to pursue the areas I am very interested and my dream will become true. This thesis is dedicated to my father and my mother.
# TABLE OF CONTENTS

ABSTRACT.................................................................................................................................................. 1

PART I: LITERATURE REVIEW.................................................................................................................. 3

1.1- Asthma .................................................................................................................................................. 3
   Definition .................................................................................................................................................. 3
   Prevalence of children with asthma ........................................................................................................ 6
   Risk factors for asthma in children .......................................................................................................... 8
   Factors inducing asthma exacerbations .................................................................................................... 20
   Physiopathology of asthma .................................................................................................................... 26

1.2- Asthma and Tobacco smoke exposure ................................................................................................. 41
   Introduction .............................................................................................................................................. 41
   Tobacco smoke ....................................................................................................................................... 41
   Active smoking and asthma .................................................................................................................... 46
   Passive smoking with asthma .................................................................................................................. 49
   Inflammation in smokers with asthma ................................................................................................... 56
   Airway inflammation in subjects with asthma who are exposed to ETS .............................................. 56
   Airway hyperresponsiveness .................................................................................................................. 56
   Airway remodelling ................................................................................................................................. 57
   Treatment ................................................................................................................................................ 58

PART II- METHODS.................................................................................................................................... 64

2.1-Clinical methods .................................................................................................................................... 65
   Questionnaires ........................................................................................................................................ 65
   Objective measurements .......................................................................................................................... 68

2.2-Laboratory methods ............................................................................................................................. 75

2.3-Data management and analysis ........................................................................................................... 81

2.4- Ethical considerations ......................................................................................................................... 83

PART III – RESULT .................................................................................................................................... 85

Chapter I
Comparison in airway inflammatory markers between healthy children and children with asthma .......................................................................................................................... 85

   Introduction ............................................................................................................................................... 85
   Methods .................................................................................................................................................... 87
TABLE OF TABLES

Table 2.1.1: Asthma medications were withheld prior to their appointments ..........74
Table 2.2.1: Cell morphology ....................................................................................78
Table 2.2.2: Results and interpretations....................................................................80
Table 3.1.1: Demographic characteristics of healthy children and children with asthma 92
Table 3.1.2: Asthma control score in children with asthma ........................................93
Table 3.1.3: Characteristics of atopy in healthy children and children with asthma ....94
Table 3.1.4: Influence of subject characteristics on FeNO ........................................96
Table 3.1.5: Association between FeNO levels and atopic sensitization .................102
Table 3.1.6: Influence of subject characteristics on EBC pH ....................................108
Table 3.1.7: Induced sputum cell counts in the healthy children and children with asthma ....110
Table 3.1.8: Influence of subject characteristics on % sputum eosinophils ..............113
Table 3.1.9: Influence of subject characteristics on sputum neutrophils ..................117
Table 3.1.10: Correlation between FeNO levels and sputum results .......................120
Table 3.1.11: Comparison in sputum cytokines between healthy children and children with asthma .................................................................126
Table 3.1.12: Correlation between IL-8 with lung function and sputum cells ............127
Table 3.2.1: Demographic characteristics of children with eosinophilic and paucigranulocytic asthma ..........................................................155
Table 3.2.2: Clinical characteristics of children with eosinophilic and paucigranulocytic asthma over the previous 12 months ..........................................156
Table 3.2.3: History of children with asthma ............................................................157
Table 3.2.4: Asthma control in the past 1 week in children with eosinophilic asthma and paucigranulocytic asthma .........................................................159
Table 3.2.5: Clinical asthma pattern between PGA and EA group .......................161
Table 3.2.6: Asthma treatment between children with EA and PGA .......................161
Table 3.2.7: Atopic status in children with eosinophilic and paucigranulocytic asthma 162
Table 3.2.8: Lung function of children with eosinophilic and paucigranulocytic asthma 163
Table 3.2.9: Comparison in AHR between children with eosinophilic and paucigranulocytic asthma ..........................................................164
| Table 3.2.10: pH of EBC in children with eosinophilic and paucigranulocytic asthma | 165 |
| Table 3.2.11: Induced sputum cell counts in children with eosinophilic asthma (EA) and paucigranulocytic asthma (PGA) | 166 |
| Table 3.2.12: Comparison in sputum IL-8 between children with asthma with the different airway phenotypes | 167 |
| Table 3.2.13: Operating characteristics of tests for eosinophilic asthma in children | 168 |
| Table 3.3.1: Characteristics of parental smoking in children with asthma | 193 |
| Table 3.3.2: ETS exposure in children with asthma by urinary cotinine | 194 |
| Table 3.3.3: Relationship between parents reported smoking and ETS exposure in children with asthma | 195 |
| Table 3.3.4: Correlation between smoking location and ETS exposure in children with asthma | 195 |
| Table 3.3.5: Demographic characteristic of children with asthma with and without parental smoking | 196 |
| Table 3.3.6: Clinical characteristics of children with asthma living with and without parental smoking | 197 |
| Table 3.3.7: History of children with asthma | 199 |
| Table 3.3.8: Asthma triggers | 200 |
| Table 3.3.9: Asthma control score | 202 |
| Table 3.3.10: Clinical pattern of children with asthma with and without parental smoking | 203 |
| Table 3.3.11: Treatment in children with asthma living with and without parental smoking | 205 |
| Table 3.3.12: Exhaled carbon monoxide in children with asthma living with and without parental smoking | 206 |
| Table 3.3.13: Atopic status in children with asthma with and without parental smoking | 206 |
| Table 3.3.14: Lung function of children with asthma with and without parental smoking | 208 |
| Table 3.3.15: AHR in childhood asthma with and without smoking parents | 208 |
| Table 3.3.16: Induced sputum cell counts in children with asthma with and without parental smoking | 210 |
Table 3.3.17: Comparison in sputum IL-8 between childhood asthma living with and without parental smoking........................................................................................................212
Table 3.3.18: Asthma control score in children with asthma with and without ETS exposure .............................................................................................................................................214
Table 3.3.19: Comparison in exhaled Carbon monoxide in children with asthma with and without ETS exposure........................................................................................................215
Table 3.3.20: Comparison in FeNO levels in children with asthma with and without ETS exposure ...............................................................................................................................................215
Table 3.3.21: FeNO levels in children with asthma with different ETS exposure levels 216
Table 3.3.22: Lung function in children with asthma with and without ETS exposure 218
Table 3.3.23: AHR in children with asthma with and without ETS exposure.......................218
Table 3.3.24: pH of EBC in children with asthma with and without ETS exposure........219
Table 3.3.25: Induced sputum cell counts in children with asthma with and without ETS exposure ...............................................................................................................................................220
Table 3.3.26: Sputum cytokines in children with asthma with and without ETS exposure .... 222
Table 3.3.27: Clinical symptoms and airway inflammatory markers in children with asthma without ETS exposure at any visits: Non-Exposed.................................................................224
Table 3.3.28: Clinical symptoms and airway inflammatory markers in children with asthma with ETS exposure at all visits: Constant Exposure.................................................................225
Table 3.3.29: Asthma control score in children with asthma who had changed ETS exposure condition from negative to positive between previous and later visits ..............226
Table 3.3.30: Exhaled CO in each child with asthma with and without ETS exposure .......227
Table 3.3.31: Induced sputum cell counts in children with asthma at with different ETS exposure periods ...............................................................................................................................................232
Table 3.3.32: Summary of the effects of ETS exposure on asthma markers.....................238
Table 3.3.33: Correlation between ETS exposure and pattern of airway inflammation.....239
Table 3.4.1: Characteristics of parents of children with asthma in Newcastle and Hanoi .268
Table 3.4.2: Characteristics of ETS exposure in children with asthma in Newcastle and Hanoi ...............................................................................................................................................270
Table 3.4.3: Knowledge of parents of children with asthma about passive smoking........273

Table 3.4.4: Attitudes of parents of children with asthma towards passive smoking........274

Table 3.4.5: Attitudes of smoking parents of children with asthma to prevent ETS exposure in children .................................................................276
TABLE OF FIGURES

Figure 1.1.1: Mediators derived from eosinophils.................................................................28
Figure 1.2.1: Interactions between asthma and cigarette smoking .............................................48
Figure 3.1.1: Median FeNO levels (ppb) in healthy children and children with asthma .....95
Figure 3.1.2: Correlation between FeNO levels and age in children with asthma .................97
Figure 3.1.3: Correlation between FeNO levels and height of children with asthma .............97
Figure 3.1.4: Correlation between FeNO levels and weight of children with asthma ..........98
Figure 3.1.5: Correlation between FeNO levels and FEV\textsubscript{1} % predicted in children with asthma ..................................................................................................................................98
Figure 3.1.6: Correlation between FeNO levels and FEV\textsubscript{1}/FVC ratio in children with asthma ..................................................................................................................................................99
Figure 3.1.7: The relationship between median FeNO levels and atopy ......................................101
Figure 3.1.8: The relationship between median FeNO levels and dust mite sensitization ....102
Figure 3.1.9: Correlation between FeNO levels and the size of dust mite reactions ...........104
Figure 3.1.10: Relationship between FeNO levels and the number of positive skin prick test reactions ..................................................................................................................................................105
Figure 3.1.11: Comparison in FeNO levels in children with asthma with and without AHR ..106
Figure 3.1.12: The relationship between FeNO levels and PD\textsubscript{15} in children with asthma ..107
Figure 3.1.13: Induced sputum in healthy children .................................................................111
Figure 3.1.14: Induced sputum in asthma ................................................................................112
Figure 3.1.15: Relationship between % sputum eosinophils and % FEV\textsubscript{1}/FVC ratio in children with asthma ..................................................................................................................................................114
Figure 3.1.16: Relationship between sputum eosinophils and atopy in healthy children and children with asthma ........................................................................................................................................115
Figure 3.1.17: Sputum eosinophils in children with asthma with and without AHR ..........116
Figure 3.1.18: Correlation between % sputum eosinophils and PD\textsubscript{15} in children with asthma ..................................................................................................................................................116
Figure 3.1.19: The success rate of the non-invasive techniques to investigate airway inflammation in children ........................................................................................................................................119
Figure 3.1.20: The correlation between FeNO levels and % sputum eosinophils in healthy children ...............................................................................................................................121
Figure 3.1.21: The correlation between FeNO levels and % sputum neutrophils in healthy children ...............................................................................................................................121
Figure 3.1.22: The correlation between FeNO levels and absolute sputum neutrophils in healthy children........................................................................................................................................122
Figure 3.1.23: The correlation between FeNO levels and % sputum eosinophils in children with asthma ........................................................................................................................................123
Figure 3.1.24: Correlation between FeNO levels and absolute sputum eosinophils in children with asthma ........................................................................................................................................123
Figure 3.1.25: Correlation between % sputum eosinophils and EBC pH in children with asthma ........................................................................................................................................124
Figure 3.1.26: Correlation between % sputum neutrophils and EBC pH in children with asthma ........................................................................................................................................125
Figure 3.1.27: Correlation between FeNO levels and EBC pH in healthy children........125
Figure 3.1.28: Correlation between FeNO levels and EBC pH in children with asthma ...126
Figure 3.1.29: Correlation between IL-8 and % sputum neutrophils in children with asthma ........................................................................................................................................128
Figure 3.2.1: The stability of sputum eosinophils and neutrophils in children with eosinophilic pattern........................................................................................................................................153
Figure 3.2.2: The stability of sputum eosinophils and neutrophils in children with paucigranulocytic pattern........................................................................................................................................154
Figure 3.2.3: Asthma triggers in children with eosinophilic asthma (EA) and paucigranulocytic asthma ........................................................................................................................................160
Figure 3.2.4: The median of fractional exhaled Nitric Oxide in children with eosinophilic asthma (EA) and paucigranulocytic asthma (PGA) ........................................................................................................................................165
Figure 3.3.1: Asthma triggers in children with asthma living with and without parental smoking ........................................................................................................................................201
Figure 3.3.2: Clinical pattern of childhood asthma with and without parental smoking....203
Figure 3.3.3: Parental smoking in childhood asthma, by clinical asthma pattern ..........204
Figure 3.3.4: Fractional exhaled Nitric Oxide in children with asthma living with and without parental smoking.................................................................207
Figure 3.3.5: pH of EBC in children with asthma with and without parental smoking......209
Figure 3.3.6: Percentage of ETS exposed children by age ........................................213
Figure 3.3.7: FeNO levels in children with asthma with and without ETS exposure.......216
Figure 3.3.8: FeNO levels in children with asthma with different ETS exposure levels ...217
Figure 3.3.9: Comparison in FeNO levels in children with asthma with and without ETS exposure ..............................................................................................................................227
Figure 3.3.10: Comparison in FeNO levels in children with asthma with low levels of ETS exposure and non ETS exposure..............................................................................................228
Figure 3.3.11: Changing in FEV\textsubscript{1} in children with asthma with and without ETS exposure ..............................................................................................................................229
Figure 3.3.12: Change in FEV\textsubscript{1}/FVC ratio in children with asthma with and without ETS exposure ..............................................................................................................................230
Figure 3.3.13: Comparison in pH of EBC of children with asthma with and without ETS exposure ..............................................................................................................................231
Figure 3.3.14: Sputum neutrophils in children with asthma with and without ETS exposure ..............................................................................................................................233
Figure 3.3.15: Comparison in absolute sputum neutrophils in children with asthma with and without ETS exposure ..............................................................................................................................234
Figure 3.3.16: Comparison in % sputum eosinophils in children with asthma with and without ETS exposure ..............................................................................................................................235
Figure 3.3.17: Comparison in absolute sputum eosinophils in children with asthma with and without ETS exposure ..............................................................................................................................236
Figure 3.3.18: Comparison in sputum IL-8 in children with asthma with and without ETS exposure ..............................................................................................................................237
Figure 3.4.1: Distribution of Fagerstrom test for Nicotine Dependence (FTND) scores of smoking parents of children with asthma in Newcastle.................................................271
Figure 3.4.2: Distribution of Fagerstrom test for Nicotine Dependence (FTND) score of smoking parents of children with asthma in Hanoi .................................................................272
ABBREVIATIONS

ACS: Asthma control score
ABS: Australian bureau of statistics
AHR: Airway hyperresponsiveness
AI: Airway inflammation
APCs: Antigen presenting cells
ASM: Airway smooth muscle
ATS: American Thoracic Society
BAL: Bronchoalveolar lavage
BMI: Body mass index
CO: Carbon monoxide
C2R: Chromatrole 2R
CysLTs: Cysteinyl leukotrienes
DNA: Deoxyribonucleic acid
DP: Dermatophagoides pteronyssinus
DRR: Dose response ratio
DRS: Dose response slope
DTT: Dithiothreitol
EA: Eosinophilic asthma
EBC: Exhaled breath condensate
ECP: Eosinophil Cationic Protein
ED: Emergency department
EDN: Eosinophil derived neurotoxin
EIA:  Exercise induced asthma
EIB:  Exercise induced bronchoconstriction
ENO:  Exhaled Nitric oxide
EPO:  Eosinophil peroxidase
EPX:  Eosinophil protein X
ETS:  Environmental tobacco smoke
FeNO:  Fractional exhaled Nitric oxide
FEV₁:  Forced expiratory volume in 1 second
FTND:  Fagerstrom test for Nicotine Dependence
FVC:  Forced vital capacity
GM-CSF: Granulocyte –macrophage colony-stimulating factor
GP:  General practitioner
GRs:  Glucocorticoid receptors
HDAC: Histone deacetylases
HDM: House dust mite
ICS: Inhaled corticosteroids
IFN: Interferon
IL: Interleukin
ISAAC: International Study of Asthma and Allergies in Childhood
cNOS: constitutive Nitric oxide synthases
iNOS: inducible Nitric oxide synthases
IQR: Inter-quartile range
LAK: Lymphokine-activated killer
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPR</td>
<td>Late-phase reactions</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>MBP</td>
<td>Major basic protein</td>
</tr>
<tr>
<td>MGA</td>
<td>Mixed granulocytic asthma</td>
</tr>
<tr>
<td>MGG</td>
<td>May Grunwald Giemsa</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>NA</td>
<td>Neutrophilic asthma</td>
</tr>
<tr>
<td>NAC</td>
<td>National Asthma Council</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthases</td>
</tr>
<tr>
<td>NO₂</td>
<td>Nitrogen dioxide</td>
</tr>
<tr>
<td>NOx</td>
<td>Nitrogen oxides</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor-κB</td>
</tr>
<tr>
<td>O₃</td>
<td>Ozone</td>
</tr>
<tr>
<td>OR</td>
<td>Odd ratio</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
</tr>
<tr>
<td>PC₂₀</td>
<td>Provocative concentration resulting in a 20% fall in FEV₁</td>
</tr>
<tr>
<td>PD₁₅</td>
<td>Provocative dose of hypertonic saline causes to fall FEV₁ by 15% of the base line</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td>PEF</td>
<td>Peak expiratory flow</td>
</tr>
<tr>
<td>PGA</td>
<td>Paucigranulocytic asthma</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
</tbody>
</table>
PM: Particulate matter
PUFAs: Polyunsaturated fatty acids
pH: Ploition hydrogen Ion concentration
RANTES: Regulated on activation normal T cell expressed and secreted
RSV: Respiratory syncytial virus
SD: Standard deviation
SO2: Sulphur dioxide
SPT: Skin prick test
TCC: Total cell counts
TNF-α: Tumor necrosis factor α
UK: United Kingdom
US: United States
WHO: World Health Organization
ABSTRACT

Airway inflammation is a key feature of asthma. Currently, airway inflammation can be detected through both invasive and non-invasive methods. Non-invasive methods are safe, feasible and a potentially useful way to assess airway inflammatory markers in both healthy children and children with asthma. In this thesis, a variety of non-invasive markers (induced sputum, exhaled nitric oxide, and exhaled breath condensate) was used to investigate childhood asthma. The aim of the first study was to compare and contrast the different airway markers between healthy children and children with asthma. The second study described the different airway inflammatory phenotypes in children with asthma, and examined clinical predictors of these phenotypes; whereas the third study investigated the effects of environmental tobacco smoke (ETS) exposure on airway inflammation in childhood asthma. The final study assessed the knowledge and attitudes of parents of children with asthma towards passive smoking.

The studies used both cross-sectional and longitudinal designs. Children with stable asthma aged between 7 - 17 years underwent clinical assessment, spirometry, exhaled nitric oxide (FeNO), exhaled breath condensate and sputum induction. Urinary cotinine was assayed to assess tobacco smoke exposure.

These studies have found that children with asthma show differences in both clinical pattern and pathological pattern compared to healthy children. These differences were apparent with elevated FeNO and sputum eosinophils. In children with asthma, there was
heterogeneity of airway inflammation. There were 2 stable inflammatory patterns: eosinophilic asthma and paucigranulocytic asthma. Unlike adult asthma, these phenotypes have different clinical features, which may facilitate detection of the phenotypes in clinical practice.

ETS exposure in children with asthma was common and associated with a non-eosinophilic pattern of airway inflammation. In children who had a change in ETS exposure, sputum eosinophils were decreased whereas sputum neutrophils were increased during ETS exposure compared to a non-ETS exposure period. Fractional exhaled nitric oxide levels were decreased after exposure to ETS compared to those at the time of non-ETS exposure. The severity of asthma was increased in children living with parents who smoked. As a result, parents of children with asthma, especially smoking parents should be more aware about the harmful effects of smoking on their children’s health and themselves. Health risk awareness about tobacco smoke helps parental smokers alter their smoking behavior as well as protecting children from ETS exposure.

In conclusion, the important findings of this thesis are the description of the inflammatory phenotypes in childhood asthma, the identification of clinical predictors of these phenotypes and the determination of the effects of ETS exposure on airway inflammatory patterns in childhood asthma. These results should facilitate understanding and management of childhood asthma and prompt treatment studies based on markers of airway inflammation.
PART I: LITERATURE REVIEW

1.1- ASTHMA

Definition

Asthma definition

The word asthma derives from the Greek word meaning “wind” or “to blow”, and describes a pattern of symptoms including wheeze, cough, chest tightness, and dyspnoea associated with variable or reversible airflow obstruction (1). However, no single clinical symptom or laboratory test is ‘diagnostic ‘of asthma. There have been several attempts to reach a consensus in the definition of asthma that covers the clinical, epidemiological and physiopathological aspects of the condition.

The first attempt to define asthma was reported at a 1959 conference (2). The consensus was:

“Asthma refers to the condition of subjects with widespread narrowing of the bronchial airways, which changes its severity over short periods of time either spontaneously or under treatment, and is not due to cardiovascular disease”.

Until the end of the 1970s, the role of airway smooth muscle as a cause of bronchoconstriction in asthma had been emphasized in many studies. The link between airway smooth muscle and airway hyperresponsiveness (AHR) was then recognized (3), and subsequently the concepts of airway inflammation (AI) and AHR were added to characterize asthma (4).
In 1993, a new definition of asthma was proposed and agreed by the Global Initiative for Asthma, under the auspices of the US National Institutes of Health and WHO (5). It states that:

“Asthma is a chronic inflammatory disorder of the airway in which many cells play a role, in particular mast cells, eosinophils, and T lymphocytes. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough, particularly at night and/or in the early morning. Those symptoms are usually associated with widespread but variable airflow limitation that is at least partly reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli”.

Although the new definition remains in use and includes both clinical and pathological characteristics of asthma, the new definition does not fully cover all of the histopathological changes in asthma. For example, airway inflammation may be not found in some people with asthma, and remodelling of the airway walls is now recognized as a key feature in asthma. In addition, the specificity of some components of the definition is limited. For example, AHR does not exist only in asthma, and can occur in subjects with allergic rhinitis who do not have asthma (6). Conversely, airway responsiveness may be normal in some patients with asthma (7).
The definition of asthma has been applied in the three main contexts (3):

+ Clinical: Asthma is an airway disorder that makes the airways more sensitive in response with a wide variety of provoking stimuli, resulting in airway narrowing, and causing asthma symptoms.

+ Epidemiological: Asthma is a combination of symptoms of asthma in the past year (including wheeze, cough, shortness of breath, dyspnoea), and AHR measured by direct (histamine, methacholine) or indirect provokers (hypertonic saline, mannitol, exercise).

+ Pathological: Asthma is a chronic airway disorder in which many cells play a role (eosinophils, mast cells, neutrophils, macrophages, lymphocytes, epithelial cells) and there is thickening and remodelling of the tissues of the airway wall.

**Definition of other relevant components**

**Wheezing**

Wheezing is an important clinical symptom of asthma. The obstruction of airflow by narrowed airways causes wheezing. Wheezing is mainly produced by narrowing of the large airways, but can also be caused by narrowing of the small airways (3). However, small airway obstruction may generate wheezing by a different way. Wheezing may result from large airways even when the disease is focused on the small airways, because of dynamic compression of the large airways by positive pleural pressure being generated to overcome the added resistance caused by the small airway obstruction (8).
Airway hyperresponsiveness

Airway hyperresponsiveness (AHR) is defined as an increase in response of the airways to a wide variety of specific and non-specific stimuli, that leads to airway constriction (3). AHR is common in asthmatic patients, but can also occur in healthy people (9, 10).

Atopy

Atopy is the production of abnormal amounts of IgE antibodies in reaction to contact with aeroallergens. Atopy can be assessed by skin prick tests with common aeroallergens. A skin prick test response of $\geq 3$ mm defines subjects with atopy (3).

Prevalence of children with asthma

According to the International Study of Asthma and Allergies in Children, the prevalence of childhood asthma ranges from 3% to 20% in different countries in the world (11, 12). In general, English-speaking countries or countries in the coastal, temperate, and subtropical areas have the highest prevalence of childhood asthma (11-13). In contrast, the developing countries or countries in the tropical areas seem to have a lower prevalence of childhood asthma (14-16). According to Woolcock, 27% of Australian children had current wheeze in 1997 (17).

The prevalence of asthma in children in the different age groups has been reviewed using the results of the standardized ISAAC questionnaires. Prevalence of asthma in 6-7 year children ranged from 4% to 32% in different countries. While asthma prevalence was higher in some countries such as New Zealand, Canada, Costa Rica, Brazil, it was lower
in others. The surveys confirmed that the prevalence of asthma in 6-7 year olds was high in English-speaking countries and Latin American countries (12, 18).

The prevalence of asthma in children aged 13-14 years was also different between countries, and ranged from 2% to 26%. Asthma prevalence was low in developing countries and Eastern Europe (14, 19) whereas it was high in Latin American and English speaking countries (13, 18, 20). Ethnic differences do not fully explain the differences in asthma prevalence. There is evidence that migration from a country of low asthma prevalence to a high prevalence country leads to a rise in asthma that approaches the rate in their adopted country (21). This suggests a key role for environmental factors influencing asthma prevalence.

The reason for different prevalence of asthma in children between countries is not explained by current knowledge of asthma pathophysiology. Researchers have suggested some hypotheses to explain this issue. As mentioned above, the definition used and methods used to diagnose asthma may lead to differences in the prevalence of asthma between countries. However, it is undeniable that prevalence of childhood asthma in Western countries is significantly higher than developing countries or Eastern Europe. Studies report that Western life style is associated with a high of prevalence of asthma (22). In fact, the prevalence of childhood asthma is also different between cities and rural areas in developing countries. Others have suggested that the different stages of westernization might lead to different asthma prevalence rates in children (23, 24).
Asthma is one of the most common chronic diseases in children in Australia. It affects up to 1 in 4 children and 1 in 7 adolescents (25). In the cross-sectional surveys conducted in children aged 8-10 years in the 11 year period (1982 -1992) in Belmont and Wagga Wagga (New South Wales, Australia), studies found an increase in prevalence of doctor-diagnosed asthma in both areas: more than fourfold (from 9.1% to 37.7%) in Belmont and twofold in Wagga Wagga (from 12.9% to 29.7%) (26). Further study in Wagga Wagga in 1992 -1997 in children aged 8-11 years showed a continued increase in diagnosed asthma (38.6%) (27). These findings suggested an increasing burden of asthma in Australian children in the last decades of 20th century.

**Risk factors for asthma in children**

Risk factors for asthma development are identified as the biological factors that predispose individuals to develop asthma, or environmental factors that induce asthma in predisposed individuals (28). Many risk factors have been identified as influencing the development of childhood asthma. However, recent studies show that the effect of gene-environment interaction on the development of immune responses in the early years of life plays a key role in the development of asthma (29, 30).

**Gender**

The effects of gender on the development of asthma have been demonstrated in many studies. Studied found that the prevalence of asthma and wheezing in small children was higher in boys than in girls (31, 32). In 2000, Cagney et al conducted a study in 2020 children aged 5-14 years in Western Sydney – Australia and found the odds for a male to
develop asthma was 1.5 times compared to a female (32). However, among adults and adolescents, females have been shown to be at more risk of developing asthma (33, 34). According to Soto-Quiros et al, at age of 6-7 years, a history of ever wheezing (p = 0.001), current wheeze (p = 0.01) and physician diagnosis of asthma (p = 0.002) have more frequent in boys than girls, but girls had more respiratory symptoms than boys (p < 0.005)(23).

Race

Some racial groups are more prone to develop asthma than others. For instance, Maori children had higher rates of diagnosed asthma and reported asthma symptoms than Pacific children in New Zealand (12). In Los Angeles, the prevalence of asthma in black, Caucasian, Asian and Latino children less than 17 years was 15.8%, 7.3%, 6% and 3.9%, respectively (p<0.001) (35).

However, studies in Chinese children living in different countries showed different asthma rates. The prevalence of asthma in Chinese children living in China was lower in comparison with those living in Western countries. It ranged from 3% to 12% up to studies and areas of China (36-38). However, the rate of asthma in Chinese immigrant children in the US was quite high (27%), same as the overall rates of childhood asthma in the US (21). A study in Melbourne in 1991 found that the prevalence of wheeze or asthma in Asian immigrants was lower than Australia –born Asian and Australia –born non-Asian children (P<0.001). However, the prevalence of asthma in Asian immigrants increased significantly with the length of stay in Australia (39). The change in the rate of
asthma prevalence when people change countries suggests that environmental factors play a major role in the development of asthma.

*Atopy*

Atopy is considered as a potent predictor for the development of asthma (40-42). Children with asthma manifest a variation of the clinical features of atopy such as eczema, rhinitis, a positive skin test or raised serum levels of total immunoglobulin E (IgE) and specific IgE to allergic agents (40). A total of 60% of adults and 80% of children with asthma have positive skin-prick tests for environmental allergens (43). A study in 662 children aged 13 years in New Zealand demonstrated that all children with diagnosed asthma and AHR were atopic subjects (44). Children who were diagnosed as atopic by skin prick test (SPT) at 4 years of age were significantly more likely to be diagnosed with asthma at 10 years of age (OR= 6.96, p<0.001)(45).

Studies also found that the severity of asthma and the persistence of wheezing were strongly related to atopy (40, 46). The presence of an atopic condition in childhood was associated with an increase in severe asthma in later life (OR= 1.66, 95% CI: 1.17 to 2.36 in the case of eczema; OR = 1.39, 95% CI: 1.1-1.92 for hay fever; and OR = 2.25, 95% CI: 1.49-3.39 for positive skin tests) (47). Children with atopy were four times more likely suffer from persistent wheezing up to the age of twenty compared to those without atopy (48).
Children with atopy have a high risk of having AHR. Atopic children diagnosed by SPT at 4 years of age had a 5-fold higher risk of AHR at 10 years of age (OR = 5.38; 95% CI: 3.06 - 9.47, p<0.001). In addition, four year old children with eczema were also at elevated risk of developing AHR at 10 years (OR = 2.08, 95% CI: 1.26-3.41; p=0.003) (45).

Children with asthma are more likely to be sensitive to indoor aeroallergens such as house dust mites, cockroaches, domestic animals and mould spores. Outdoor allergens such as pollen, mould spores may also contribute to development of asthma in children (49, 50).

Dust mite allergen exposure is considered as a major risk factor for the development of pediatric asthma and asthma attacks (49, 51, 52). Most children with asthma are sensitive to house dust mite (HDM)(53). In Australia and New Zealand, the principal dust mite is *Dermatophagoides pteronyssinus* (*Dp*) (54). Children exposed to high levels of house dust mite during the first year of life are at increased subsequent risk of sensitization and the development of asthma (55). The quantity of HDM is strongly associated with the prevalence of dust mite sensitization. In places where humidity is low, the levels of HDM are low, and there is a low prevalence of dust mite sensitization (56, 57). On the other hand, in hot and humidity places, mite allergen levels are high; the prevalence of HDM sensitization is high, and associated with high prevalence of asthma (58, 59). Children with asthma living on the coast are more sensitive to HDM than children living in land. Peat *et al* conducting a study in New South Wales in 1993 showed that the adjusted odds
ratios for current asthma in children sensitized to house dust mites were 21.3 in the coastal region and 2.7 in inland (49). Sensitization with HDM was also strongly associated with AHR (37, 60), and predicted the persistence of wheezing (61, 62).

While there is some evidence in support of link between HDM exposure and the development of asthma and allergies, there is also evidence that this may be an epiphenomena rather than a direct causal factor. A study in newborns at high risk of developing allergies found that HDM avoidance did not show a protective effect on the development of sensitization to HDM at age 24 months (63). The Manchester studies suggested that there was little evidence to support the use of mite-proof encasings as a single intervention to avoid the development of asthma and allergies in adults. However, single or multifaceted interventions in children may bring some benefit (64).

Grass pollen is also recognized as a major allergen related to allergic rhinoconjunctivitis and asthma. It is the second most common allergen, after HDM associated with the development of asthma (50). Asthma symptoms, AHR, and medication use increased during and fell after the grass pollen season in children who were sensitized to mixed grass pollen (65).

Cockroaches are ubiquitous and highly allergenic. Cockroach allergens are found in the body and faecal droppings of both German and American species (54). In USA, high cockroach allergen levels have been seen in older inner-city dwellings and in tropical climates (66). De Vera et al found that children living in the inner-city who were
sensitized to cockroaches were significantly more likely to have previous episodes of wheezing (67). Children who were exposed to cockroaches in their infancy were 2 times more likely to have a diagnosis of asthma at 5 years of age than those were not exposed (68). The evidence suggests that cockroach is another important indoor allergen contributing to asthma.

Some fungi such as *Alternaria* have been found to be risk factor for the development of asthma (69). Sensitization to *Alternaria* was related to increased prevalence of the current wheezing (OR=3.3, 95% CI: 1.1-11) (62). A study in Australia demonstrated that the odds ratios for current asthma in children sensitized to *Alternaria* were 3.4 (95% CI: 1.3-9.1) on the coast and 5.6 (95% CI: 3.1-10.1) inland (49). In children with asthma, being sensitized to *Alternaria* were significantly associated with AHR (49).

*Family history*

Family history of atopy and asthma are important risk factors in the development of asthma in children (70, 71). A study in 3024 parents and their children found that the prevalence of asthma, allergic rhinitis and eczema among parents has similar to that in their children (71). A family history of allergy was demonstrated in 90% of children with asthma (72). Maternal allergy was a strong risk factor for asthma and allergic diseases in children (73).

The odds ratio for the development of asthma in children who have a family history of asthma ranges between 2 - 27 (74-76). In children sensitized to inhaled allergens, the risk for the development of asthma was increased only if their parents suffered from a history
of asthma or atopy (OR= 15.56; 95% CI: 5.78-41.83); the risk being strongest for maternal asthma (77).

Parental and/or sibling history of asthma and allergy are strongly associated with early-onset persistent asthma. The odds ratios for early-onset persistent asthma, early-onset transient asthma and late-onset asthma in children where both parents had asthma were 12.1 (95% CI = 7.91-18.7); 7.51 (95% CI = 2.62-21.5) and 5.38 (95% CI = 3.40-8.50), respectively (78). The findings suggest that genetically predisposed children have a high risk for the early development of asthma.

Genetics play an important role in the development of childhood asthma, as indicated by family history. The identification of candidate genes and chromosomal regions linked to asthma risk are being investigated. There is strong evidence that an imbalance in the Th1/Th2-cell response of genetically predisposed individuals to common aeroallergens determines the pathogenesis of allergic asthma and other atopic disorders (79).

Genetic studies have reported that multiple genes are contributed to develop asthma. This region includes a cluster of cytokine genes, and genes encoding IL-3, IL-4, IL-5, IL-9, IL-13, granulocyte macrophage colony stimulating factor, and the beta chain of IL-12 (80).
Epidemiological studies suggest a relationship between childhood infection and asthma, reporting both increased and reduced risk of the development of asthma in children. Studies reported that some infections may promote a Th1 host response which protects from the development of asthma and allergies in children (81). However, whether respiratory viruses could generate a protective effect is still debated. Both the respiratory system and the immune system undergo rapid maturation during the first year of life. Certain viruses have been implicated in the inception of the asthmatic phenotype. Infections by respiratory viruses in early life may affect the immune system, resulting in modification of immune responses, and subsequent development of allergy and asthma (82). However, the mechanism by which early childhood infections might influence the developing immune system is quite complex. For instance, viral infections could adversely affect lung development, leading to structural lung changes and functional deficits at an early age of children with asthma (83).

Several studies suggest that infection with Respiratory Syncytial Virus (RSV) in infancy is associated with the development of asthma later. A cohort study of children from birth to 7.5 years of age demonstrated an increase in asthma frequency and allergic sensitization among those with RSV bronchiolitis compared to those without RSV bronchiolitis. The prevalence of asthma in the RSV group was 23% compared with 2% in the control group \( (P < 0.001) \); the prevalence of allergic sensitization was 41% in the RSV group and 22% in the control group \( (P = 0.039) \) (84). In addition, the study found that a history of RSV bronchiolitis in early of life was the most important risk factor for the
development of asthma and raised IgE antibody titers (85). The host response to RSV infection favors a Th2 immune response, which may be mediated by overexpression of IL-4 cytokines (86). In summary, RSV bronchiolitis during the first year of life is suggested as an important risk factor for the sensitization to common allergens and development of asthma after 2 years of age, particularly in children with a family history of atopy and/or asthma (85). In contrast, a Tucson study suggested that while RSV lower respiratory tract infection were associated with increased early childhood wheeze, risk of wheeze decreased markedly with age. There was also no association between RSV infection and the development of atopy (87).

The role of Rhinovirus in the development of asthma has been reported. Children with wheezing in infancy, who have suffered from rhinovirus infection have a higher risk of developing asthma than those without rhinovirus infection (OR = 4.14, 95% CI: 1.02-16.77; P=0.047)(88). Rhinovirus can infect not only in the upper but also in the lower airways. Bronchial and pulmonary epithelia cells infected by rhinovirus release rich sources of inflammatory mediators, which may initiate or stimulate airway inflammation and obstruction. In an atopic environment, responses to the Rhinovirus are skewed toward the development of Th2 response, which may further enhance the development of asthma (89).

The development of immunity in newborns was noted by the “hygiene” hypothesis. T helper cells can be subdivided into two main phenotypes, Th1 and Th2, based on their pattern of cytokine production. A balance between Th1 and Th2 responses is important in the proper regulation of immune responses. It is possible that some respiratory viral
infection in the early year of life affect on the regulation and development of immunity, that leads to imbalanced levels between Th1 and Th2 cells in later life, which stimulate young children to remain prone to the development of Th2 response, causing atopic disease and asthma development (90, 91).

*Environmental tobacco smoke (ETS) exposure*

Passive smoking has been shown to a risk factor for the development of atopy and asthma in children (92, 93). Prenatal exposure to ETS has been associated with impaired lung function and increased risk of developing asthma in children (94, 95). Both prenatal and postnatal exposure to ETS may induce bronchial hyperresponsiveness in children (96, 97). Zejda linked the prevalence of cumulative incidence of childhood asthma with the number of smokers in family: from 2.0% in non-smoking families to 4.2% in one-parent-smoking families and 5.4% in two-parent-smoking families (98). Maternal smoking seemed to be a higher risk for development of asthma than other smoking (99). Maternal smoking was significantly associated with a subgroup of childhood asthma with negative skin prick tests (100).

Interestingly, a study in Sweden found that ETS exposure during childhood may affect the prevalence of asthma in adults. In adult never-smokers, the prevalence of physician-diagnosed asthma in subjects with childhood ETS exposure was 7.6% versus 5.9% in subjects without childhood ETS exposure (p = 0.036). In never-smokers without a family history of asthma, the prevalence of physician-diagnosed asthma in subjects reporting childhood ETS exposure was 6.8% versus 3.8% in non-exposed subjects (p < 0.001) (101). These findings suggest that childhood ETS exposure is related to an increased
prevalence of asthma among adult never smokers, especially when the subjects do not have a history of asthma or allergy.

The potential mechanism by which ETS exposure is a risk factor for early childhood wheezing or asthma may involve genetic predisposition, impairment of lung development, and altered lung inflammatory responses (102). Recent studies have shown that the interaction between genetic alterations and environmental factors such as active or passive smoking causes the development of atopic diseases such as asthma, allergic rhinitis and atopic eczema (103). ETS can modify genetics which induces the development of asthma and asthma symptoms. Some chromosomal regions (e.g. 5q) might harbor genes that exert their effects predominantly in combination with ETS exposure (103). In subjects with asthma and AHR, the strongest evidence for linkage was observed for chromosomes 3p and 5q. The children who were exposed to passive smoking from their families showed the evidence for linkage of AHR to 5q (104). When these detoxification enzymes are genetically defect or missing by ETS, the capability of the lung to metabolize hazardous substances is impaired. As a consequence, airway inflammation may occur and the lung function may be compromised allowing allergens to penetrate and asthma to start (103). Currently, no studies report the association between passive smoking and airway remodelling in subjects with asthma.

**Diet**

Epidemiological studies suggest that diet is associated with the development of asthma. The role of breast feeding and omega-3 fatty acids in the prevention of asthma is
controversial. The risk for eczema and onset of allergy at 4 years of age was decreased if children were breast fed for 4 months or more (105). The risk of childhood asthma increased if the exclusive breast feeding was stopped before 4 months (OR= 1.28, 95% CI: 1.01-1.62; p=0.038) (106). However, other studies indicated that breast feeding did not prevent the development of asthma, delay its onset and reduce its severity (107, 108).

There is evidence that dietary modification has the potential to influence the incidence of asthma as well as the severity of the disease. Studies have found that Omega-3 polyunsaturated fatty acids (Omega 3-PUFAs), as found in fish oils, can reduce the production of inflammatory eicosanoids and cytokines. Omega 3-PUFAs can reduce mediator production both directly, by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism, and indirectly by altering the expression of inflammatory genes through effects on transcription factor activation. Omega 3-PUFAs also increase a family of antiinflammatory mediators (109) and reduce AHR (110). In contrast, omega-6 PUFAs cause the release of pro-inflammatory mediators such as Leukotrienes and Prostanoids, which are associated with inflammation in asthma (110). The typical Western diet is a possible cause for the increased prevalence of asthma in Western societies. Studies suggest that Western people consume about 20-to 25 fold more omega-6 PUFA than omega-3 PUFA (110). It is proposed that this relative excess of omega-6 PUFAs to omega-3 PUFAs can induce pro-inflammatory mediator production and may increase asthma prevalence.
While many studies suggested that fish oil supplementation may reduce the development of asthma, the results of the CAPS (Childhood asthma prevention study) in Sydney, which demonstrated no effect of fish oil on the asthma development, but some symptoms have been reduced in children with high omega-3 fatty acid levels in plasma (111).

**Outdoor air pollution**

Evidence that outdoor air pollution causes the development of asthma is unclear. One prospective study over a 15 year period in 3,000 non-smokers demonstrated that long-term exposure to ambient ozone was associated with the development of asthma in adult males (112). A comparison of the prevalence of asthma among schoolchildren in the two cities of the metropolitan region of Rio de Janeiro (Brazil) with different particulate matter 10 (PM10) concentration found that “wheeze ever” was significantly higher in the region with high PM10 concentration compared to in city with low PM10 concentration (35.1% versus 29.9% respectively; P=0.01)(113). These studies suggest that the prevalence of childhood asthma is associated to atmospheric pollutions. Currently, there are no experimental data in humans to show that outdoor air pollution causes asthma development.

**Factors inducing asthma exacerbations**

The episodes of respiratory symptoms associated with an exacerbation of asthma can occur at different times of the year, and are induced by different triggers with the inflammatory response varying among patients. Recognized triggers of asthma include viral infections, allergens, air pollution, and cigarette smoke.
**Viral infection**

Respiratory viral infections are the major factors causing exacerbation of asthma. Viruses were detected in 80% of episodes of reduced peak expiratory flow (114). Although respiratory viral infections are common in asthma exacerbations, the exact mechanism by which they induce asthma attacks is unclear (90, 115). RSV and parainfluenza viruses are the major triggers of wheezing-related respiratory infections in early childhood; rhinovirus and influenza are more common cause of asthma exacerbations in older children (82).

RSV infection is more likely to trigger severe wheezing in infants or small children who are already predisposed to wheezing and asthma than in those with low risk of wheezing or asthma (114). Rhinovirus infections are reported as the main cause of wheezing in children and adults, being responsible for about 60% of wheezing episodes (114). Interestingly, recent data suggests that rhinovirus is the predominant respiratory pathogen even in early childhood (116). Rhinoviruses may stimulate bronchial epithelial cells to produce cytokines and pro-inflammatory chemokines in *vivo* and in *vitro*. They may also stimulate the cholinergic and non-cholinergic nervous system, raise the production of ICAM-1 and increase a T-lymphocyte non-specific response. These findings suggest that Rhinovirus infections may induce asthma exacerbations by multiple cellular pathways.

**Allergen exposure**

*Indoor allergens:* There is no doubt that indoor allergens play an important role in triggering asthma symptoms. Exposure to high dust mite allergen levels can exacerbate
childhood asthma (117, 118). Positive relationships have been found between the levels of HDM exposure and the levels of AHR (119), increased asthma symptoms, and reduced peak expiratory flow in subjects with asthma who were sensitized to HDM (120). Reduction of exposure to HDM allergens has improved asthma symptoms, peak expiratory flow, FEV$_1$, AHR (121, 122), and reduced both total and specific IgE (121). By contrast, a recent Cochrane clinical trial reported that there was very little evidence that HDM allergen avoidance improved asthma outcome for patients, including asthma symptoms and the dose of ICS use (123).

Exposure to Cockroaches may lead to exacerbations of asthma and allergic rhinitis in sensitized patients (124). Exposure to cockroach allergens was associated with increased hospital visits due to asthma in inner city Chicago (125). Dust mite and cockroach are important indoor allergens that are associated with asthma, particularly in urban environments.

Outdoor allergens: The significance of outdoor allergens as causes of exacerbations in childhood asthma is less clear. An association between grass pollen, mold spore exposure and asthma ED presentations were observed in Mexico City (126). A panel study of subjects with asthma showed that exposure to fungal spores can trigger asthma attacks, causing increased asthma symptoms, decreased lung function, and increased asthma medication use (127). However, other studies found no consistent effects of pollen or mold spores on childhood asthma (128, 129). The different findings on the role of
outdoor allergens in asthma exacerbations may be due to differences in the concentration of ambient aeroallergens.

**Exercise-induced asthma (EIA)**

Exercise is a powerful trigger of asthma symptoms and reversible airflow obstruction. EIA is seen often in children and adolescents with asthma. Epidemiological studies find that exercise induced bronchoconstriction (EIB) occurs in 40% to 90% of patients with asthma (110, 130). Inflammatory mediators play a central role in EIA. The diagnosis of EIA is usually straightforward in most patients, but some patients may have atypical symptoms. Most subjects with EIA will respond to treatment with short acting beta agonists (131, 132) but some need other asthma medications, including daily inhaled corticosteroids (132).

**Environmental tobacco smoke (ETS) exposure**

ETS exposure from parents, siblings, colleagues or visitors who smoke, is associated with increased asthma symptoms, AHR, respiratory infections, asthma attacks and frequent hospitalization in children with asthma (133). Poor asthma control was observed in children with asthma who were exposed to ETS (92). Overall, parental smoking plays an important role in triggering asthma attacks in children. Parental smoking was significantly associated with wheezing, exercise-induced wheezing, nocturnal coughing, and symptoms of rhinitis in the last 12 months in children with asthma (134). Asthmatic symptoms have been shown to decline after the ETS exposure was reduced (92).
Outdoor air pollution

Outdoor air pollution is associated with asthma exacerbations in children. Studies found reduction of lung function, increased AHR and respiratory symptoms in both subjects with and without asthma who were exposed to ozone (135, 136). Asthma may be exacerbated following periods of high ozone pollution (137). Ozone exposure was also associated with increased emergency department visits due to asthma (138).

An association between short-term changes in ambient particulate matter concentrations and asthma outcomes was observed in several studies. Emergency hospital admission due to asthma attacks in children was associated with regional concentration of PM10 in Melbourne (138). A study in 5 Australian and 2 New Zealand’s cities demonstrated a correlation between outdoor air pollution and short-term increase in childhood hospital admissions due to asthma attacks in children aged 5-14 years (139).

Long-term exposure to air pollutants might cause an increased incidence of chronic cough and decreased lung function (140, 141). Increased severe asthma attacks and asthma medication use were reported in children with asthma who were exposed to high ozone levels and high PM concentrations (142).

Change in weather

The role of weather on asthma symptoms and other outcomes has been investigated with inconsistent findings. A link has been observed between exacerbations of childhood asthma and afternoon weather gradients (143). Thunderstorm-associated asthma attacks
have been reported. The studies observed that a stagnant air mass the day before the thunderstorm may cause the accumulation of spore and pollen reservoirs within mould and plant structures. At the thunderstorm, the high winds triggered a sudden release of high spores and pollens into the atmosphere, which was probably responsible for the asthma exacerbations (144). Increased barometric pressure, change in temperature, and rainfall were associated with increased asthma ED presentations (145, 146). Asthmatic symptoms were increased by low temperatures in children with asthma (147). However, Forsberg did not find any association between changes in temperature and increased asthma symptoms (148).

*Emotion*

Psychological factors are recognized to influence the onset and course of asthma. There is evidence to support the hypothesis that asthma attacks are associated with depressive and panic disorders (149, 150). The experience of pleasant and unpleasant emotions can cause elevated airway resistance in subjects with asthma (151).

Emotional events such as laughing, stress and sadness are significantly associated asthma attacks. Liangas reported that 42% of subjects with asthma had laughter - associated with asthma (152). Laughter was reported as an asthma trigger, more often seen in subjects with poor asthma control (152). However, the mechanism of laughter-induced asthma is unknown.
The preceding section identifies that asthma is common in children and reviews the known risk factors associated with asthma prevalence and asthma exacerbations. Exposure to ETS is identified as an important risk factor for the development of asthma and asthma exacerbations. The following section will review the pathogenetic features of asthma.

**Physiopathology of asthma**

Asthma is a complex inflammatory disease of the lung characterized by airway inflammation and remodelling, airway hyperresponsiveness, and variable airflow obstruction. Examination of the airways of patients who have died from asthma have found airway inflammation, sparing of the lung parenchyma, and increased airway remodelling (153). The specific changes include an infiltration of airway inflammatory cells, especially eosinophils, the presence of intraluminal mucous secretion, an increased amount of airway smooth muscle, airway epithelial desquamation and repair, and the presence of a thickened, hyalinized subepithelial basement membrane (153). Airway inflammation is accepted as a fundamental characteristic of asthma (154, 155).

*Airway inflammation*

The clinical pattern of asthma is well described in both adults and children (156). Airway inflammation is present in both allergic and non-allergic asthma (157), and in all grades of asthma severity (155, 158). The question is whether subjects with asthma with the different levels of severity have similar airway pattern. Current studies have found that there is heterogeneity of airway inflammation in asthma (159, 160), giving rise to
different in inflammatory phenotypes (161). In adults with asthma, AI is described with as an abnormal accumulation of eosinophils, neutrophils, lymphocytes, mast cells, basophils, macrophages, dendritic cells, and myofibroblasts in the airway wall. Different phenotypes can be identified based on the presence or absence of eosinophils and neutrophils (161). Interestingly, a study found that in a group of adults with stable asthma, some subjects had persistent asthma symptoms although airway inflammation (eosinophils, neutrophils) was similar with healthy subjects (161). Airway inflammatory patterns are also different between exacerbations and stable asthma (162, 163). In addition, the different asthma triggers may induce the different airway inflammatory response. For example, allergen induces an eosinophil response and viral infection induces a neutrophil response (162).

In the past, eosinophils and mast cells were suggested as having a pivotal role in the airway inflammation in asthma (164). Nowadays, studies show evidence that eosinophilic inflammation is not the only pattern involved in the inflammatory mechanisms of subjects with asthma. Current studies have identified at least two subtypes of airway inflammation in asthma, one with increased sputum eosinophils, called eosinophilic asthma and another without increased sputum eosinophils, called non-eosinophilic asthma (159, 160).

Airway phenotypes

Eosinophilic asthma
Eosinophils are granulocytes characterized by bilobed nuclei and cytoplasmic granules that stain orange-red with eosin. They are produced in the bone marrow (165). Eosinophil differentiation, like that of all leukocytes, is influenced by cytokines. Cytokines released from activated T lymphocytes, such as IL-5, promote maturation, activation, and prolonged survival of eosinophils (166).

Eosinophils are recognized as the inflammatory cells that typically characterize the airway inflammation in asthma (165). Eosinophils can produce a variety of pro-inflammatory cytokines and mediators, all of which have the potential to participate in the inflammatory process. They include basic granular proteins, some of which have enzymatic activity. Eosinophils also produce chemokines, cytokines, fibrogenic and growth factors, and lipid mediators [cysteinyl leukotrienes, LTC(4)/D(4)/E(4)] that play major roles in the pathogenesis of asthma and other forms of allergic inflammation (167, 168).

Figure 1.1.1: Mediators derived from eosinophils (Kay, 2005) (168)
Experimental studies have demonstrated that eosinophils stimulate the release of inflammatory mediators which cause contraction of airway smooth muscle, AHR, bronchial epithelial damage and airflow obstruction animal models (165, 169).

Eosinophils are seldom present in sputum samples from normal subjects. There is evidence that eosinophils are found in the peripheral blood, sputum, BAL fluid, and airway tissues of patients with asthma (155, 170). According to National Institutes of Health, sputum eosinophilia has long been associated with asthma (171).

Sputum eosinophils were significantly higher in stable asthma and in exacerbations of childhood asthma compared to healthy children (164, 172). Similarly, eosinophil numbers in BAL were significantly elevated in children with atopic asthma compared with healthy controls (173, 174). A study in wheezy preschool children found that eosinophils in endobronchial biopsies was significantly greater compared with control subjects (P < 0.05) (175). Sputum eosinophils were increased during asthma attacks, and dropped significantly two weeks later when children had recovered (172).

Sputum eosinophil numbers were positively correlated with the mean weekly total asthma symptom scores (r=0.52; p <0.01) and negatively correlated with the mean percent PEFR on waking (r= -0.50; p<0.01)(176) and negatively correlated with forced expiratory volume in one second (FEV1)(177). Childhood asthma with AHR measured by hypertonic saline was strong correlated with sputum eosinophil counts (OR: 4.36, 95% CI: 1.70- 11.2) (178). There were significantly higher sputum eosinophil counts in the
patients with severe asthma compared with those with mild or moderate asthma, but no significant difference between mild and moderate asthma was found (176, 179). These findings suggest that sputum eosinophils in the airway are closely linked to the symptoms and airflow obstruction of asthma.

While airway eosinophilia was the pathologic features of adulthood asthma, it was already observed in children with asthma (180-182), and even in children with atopy without asthma (181, 182).

Increased sputum eosinophils have been reported in up to 80% of corticosteroid-naïve asthma patients (183). Treatment with inhaled corticosteroid results in a significant decrease in eosinophil numbers and a good clinical response in asthmatic subjects with elevated sputum eosinophils (184). Treatment with oral corticosteroids in asthma exacerbations showed the same results, with a reduction in sputum eosinophils and ECP, but no effect on the sputum total cell count, neutrophils and lymphocytes (185, 186). When eosinophils are absent, then there is little response to corticosteroid in airway disease (187). In clinical trials, the withdrawal of inhaled corticosteroid led to an increase in sputum eosinophils, an increase in asthma symptoms, and changes in lung function and airway responsiveness (188).

In sumary, airway eosinophils are an integral part of the pathology of asthma. The measurement of sputum eosinophils is useful in the diagnosis asthma, in the assessment of the severity of asthma and in the management of asthma.
Non-eosinophilic asthma

The non-eosinophilic asthma (NEA) phenotype is characterized by asthma symptoms and AHR occurring in the absence of sputum eosinophilia (160). According to Douwes, only 50% of asthma cases can be contributed to by eosinophilic airway inflammation (160). Non-eosinophilic asthma is common and exists in all grades of asthma. Gibson et al investigated airway inflammation in 56 adults with persistent asthma, and a non-eosinophilic pattern of airway inflammation was observed in 59% of cases (159). This study also found that sputum neutrophil percentage, absolute neutrophil counts and IL-8 were increased in subjects with non-eosinophilic asthma. Wenzel et al also confirmed that there were at least two subtypes of airway inflammation in severe and refractory asthma based on the presence or absence of eosinophils, although they were clinically similar (189). NEA was also reported in mild asthma (190). Turner et al found during a mild asthma exacerbation, about half of subjects did not have increased sputum eosinophils (191). In adults, non-eosinophilic asthma was often associated with increased neutrophils and an acute inflammatory response involving a number of cytokines such as IL-8, and TNF-α which have a role in infiltration and activation of neutrophils in the airways (160, 192). Higher levels of neutrophils have been identified from sputum (193) and bronchial specimens in severe asthma (189). Also a higher concentration of neutrophils has been observed in the BAL of patients with severe asthma compared with mild-moderate asthma (194). A study by Anees in subjects with occupational asthma found that induced sputum macrophages were increased in subjects with NEA in comparison to those with EA during a work exposure (195).
A proportion of neutrophils > 10% in BAL was found in one-third of the children with asthma and in half of the infantile wheezers, and reflected the symptom severity (174). The activation of neutrophils was associated with preschool viral induced wheezing (196). Studies found that there was a group of older children who have severe asthma and poor response to ICS, which were suggested to correlation non-eosinophilic inflammation (197, 198). Other study observed that childhood asthma with severe symptoms and required high dose ICS did not show the increased sputum eosinophils (199).

Sputum eosinophils and AHR are characteristic of asthma. Eosinophils were significantly correlated with airway responsiveness to hypertonic saline solution (200). In subjects with asthma, the absence of sputum eosinophils may lead to less AHR in NEA compared to eosinophilic asthma (160).

Nowadays, while the mechanism of eosinophilic asthma is well described, the mechanism of non-eosinophilic asthma is not fully understood. Studies suggest that mast cell infiltration in airway smooth muscle (201) or neurogenic mechanisms may partly explain the mechanism of AHR in non-eosinophilic asthma.

In subjects with asthma, the presence of sputum eosinophilia predicts a good response to corticosteroid (202, 203). However, there is increasing evidence that subjects with NEA do not have a favorable response to corticosteroid (194, 203). Studies found that severe and refractory asthmatic patients which often responds poorly to corticosteroids demonstrate no increase in eosinophils, and instead have increased neutrophils (189).
Since the non-eosinophilic pattern of asthma is common, the treatment and management of asthma should be carefully considered because major asthma treatment currently focuses on eosinophilic asthma.

The cells that are proposed as relevant in NEA include neutrophils and macrophages. The difference in inflammatory phenotypes in children with stable asthma is not well described. An investigation of sputum cell count based on the presence or absence of eosinophils, neutrophils and macrophages is needed to identify the different airway phenotypes in children with asthma.

Sampling methods
To investigate AI in asthma, studies use both invasive and non-invasive methods. Bronchial biopsies and bronchoalveolar lavage (BAL) are invasive techniques. Non-invasive techniques are used more often to investigate AI, especially the examination of cells and mediators from induced sputum.

Bronchial biopsy technique has some limitations: small samples are collected from proximal airways, it is only performed in specific centers, and it can be difficult to conduct in children (204).

BAL is often combined with bronchial biopsy. BAL samples both cells and fluid from the lung. The sample collects only the cells and fluid present on the epithelial surface of airways and alveoli (epithelial lining fluid). The findings from BAL samples might not
reflect what is happening in the airway tissue, the principal sites of pathology in asthma (205).

Sputum induction is safe and feasible in subjects older than 6 years (206). Sputum samples can measure differential cell counts and some inflammatory mediators, but they do not completely represent AI in subjects with asthma (207). Sputum eosinophil numbers reflect the proportion of eosinophils in the total cell count collected from BAL, but not the eosinophil numbers in the tissues. However, changes in sputum eosinophil numbers might reflect the changes in eosinophil numbers in both BAL and biopsy after treatment by anti-inflammatory medication (208). Sputum predominantly samples cells and mediators from large airways.

Airway hyperresponsiveness

AHR is accepted as a fundamental characteristic of asthma. Increased airway responsiveness is usually part of the diagnosis of asthma, but not all subjects with AHR have asthma. A study of 2363 Australian school children aged 8-11 years using Histamin inhalation test found that 6.7% of subjects had AHR without symptoms or a previous diagnosis of asthma (7). AHR may be present in other diseases such as allergic rhinitis (6, 209), and obesity (210). Interestingly, AHR which was assessed by direct measurement such as histamin or methacholine was observed in healthy people (9, 10). On the other hand, 5.6% children diagnosed with asthma did not show AHR (7).
Studies have found that there are many factors that may contribute to the development of AHR in children. Atopy is a major determinant of AHR in children with or without a reported history of asthma and wheezing. In infants, the early onset of atopy may influence AHR (211). Sears showed a strong relationship between atopy and AHR, particularly in children who were sensitized to house dust mite (P<0.0001) (60).

AHR in early life is associated with lower respiratory diseases, and the development of asthma later (212). Children with a paternal history of asthma had significantly greater AHR than those without a paternal history, and the effect was greater in children with two asthmatic parents (213). These studies highlight the potential contribution of genes in the development of AHR in childhood asthma.

The mechanism of airway hyperresponsiveness

The mechanism of AHR in asthmatic subjects currently remains uncertain. In addition, the mechanisms of transient AHR and persistent AHR may be different. Many hypotheses have been suggested in order to clarify AHR mechanism in subjects with asthma.

Airway inflammation

AI and AHR are key characteristics of asthma. The question is whether AI is associated with AHR or whether they are independent characteristics in asthma. Studies suggest that AI can stimulate an increase in AHR (214, 215). Moreover, improvement in AI by
treatment also improves AHR (216). The link between AI and AHR suggests that AI might contribute to the mechanism of AHR in subjects with asthma (217).

**Mechanics**

AHR has been linked to reduced airway caliber (218, 219), increased airway wall thickness (218, 220), and increased airway permeability (221, 222). The observations suggest that the mechanism of hyperresponsiveness may be related to the geometric effects of reduced airway diameter.

**Airway smooth muscle alterations**

Airway smooth muscle (ASM) has a role in the response to many stimuli through different pathways. Alteration in the structure or function of ASM may affect airway responsiveness (223). There is exaggerated ASM contraction in response to sensitizing stimuli in subjects with asthma (224). Remodelling of ASM can involve hypertrophy (an increase in individual muscle cell size) and hyperplasia (an increase in cell number), and also contribute to cause of AHR (225, 226). Several factors have been suggested that could alter ASM, such as inflammatory mediators, growth factors, cytokines, extracellular matrix proteins, and genetic factors (225, 227).

In sum, the precise mechanism of AHR in asthma is unknown. The postulated hypotheses that suggest there is link between AI and alteration of ASM, including functional and structural changes, which may play a modulating role in AHR in subjects with asthma.
**Airway obstruction**

Airway inflammation, airflow obstruction, and AHR are mainly characteristics of asthma. In the clinic, airflow obstruction in asthma may be reversible or irreversible. Although childhood asthma often exhibits reversible airway obstruction, in some children and adults with asthma, airflow obstruction can become irreversible or only partially reversible (228, 229).

**Airway remodelling**

Longitudinal studies demonstrate an accelerated and progressive decrease of lung function over time of subjects with asthma (230, 231). Asthma is characterized by a chronic airway inflammation followed by healing, the end result of which is the alteration of airway structure and function referred to as airway remodelling. Cytological and histological changes in the airway structure might explain the decrease of lung function over time that many patients with asthma experience (231). In asthma, characteristic changes of airway remodelling include goblet cell hyperplasia, subepithelial fibrosis, increased size and number of microvessels in the submucosa, hyperplasia and hypertrophy of airway smooth muscle, and hypertrophy of submucosal glands (232, 233).

Studies have linked airway remodelling to airway inflammation, and proposed that remodelling induced by cytokines and mediators produced by inflammatory cells as part of the chronic airway inflammatory process in asthma (234). Recent studies suggest that airway smooth muscle cells might play a pivotal role in the remodelled airway (227). Changes in ASM can be induced by cytokines, growth factors, matrix proteins, cell
adhesion molecules and other potential costimulatory molecules that modulate airway remodelling (225, 227). These ASM cell functions might also directly or indirectly modulate submucosal airway inflammation and promote airway remodelling (235). In addition, an increase in ASM mass and alteration in the extracellular matrix may enhance smooth muscle growth and contribute to the mechanics of airway obstruction (236).

These changes of airway structure can occur in all levels of asthma. Goblet cell hyperplasia and subepithelial collagen deposition may appear in even mild asthma. An increase in ASM and gland volume are often present in severe asthma (234). Although airway wall thickness is variable, overall it is increased in asthmatic patients compared with healthy controls (237).

The remodelling processes accompanied with chronic inflammatory cell infiltration interact in a complex fashion and contribute to the development of airflow limitation in asthma (238). These changes lead to reduced airway caliber and may result in increased airflow resistance, particularly when subjects have bronchial smooth muscle contraction and AHR (239). Therefore, airway remodeling can significantly affect the functional characteristics of asthma, such as AHR and reversibility of airway obstruction (233, 240).

In asthma, evidence of airway remodelling is found in biopsy specimens (such as collagen deposition on basement membrane) (230). The consequences of airway remodelling include incompletely reversible airway narrowing, AHR, airway edema, and increased mucus secretion which manifest as clinical asthma symptoms such as dyspnea,
wheeze, sputum production, and susceptibility to asthma exacerbations. The changes might even contribute to death due to airway obstruction resulting from smooth muscle contraction, airway edema, and mucus plugging (234, 241). Airway remodelling is believed to cause an irreversible airflow obstruction, an increase of AHR and the severity of asthma (232, 242).

Currently, noninvasive methods to investigate airway remodelling are lacking, therefore evaluation the efficacy of asthma medication to improve airway remodelling has been difficult. However, as mechanisms of airway remodelling will be identified, specific treatment for airway remodelling will be expected (234). Thus, among anti-asthma drugs, early and long term intervention with inhaled corticosteroid may prevent progression of airway remodelling by suppressing allergic airway inflammation (232, 237, 240). Recent evidence indicates that determining the appropriate dose of inhaled steroids depends on asthma symptoms and lung function. High doses of inhaled corticosteroids can significantly reduce both inflammatory cells and some components of airway remodelling, such as the increased airway wall vascularity and the basement membrane thickness. Conversely, low doses of inhaled corticosteroids can significantly affect only on airway cell infiltration (240). In contrast, some observations fail to show any effects of inhaled corticosteroids on long-term asthma outcome. Studies in young children with a positive asthma predictive index demonstrated that two years of inhaled-corticosteroid use did not change the development of asthma symptoms or lung function during a third year of withdrawal of ICS therapy (243). A follow-up for four to six years in children
with mild-to-moderate asthma found no significant difference in lung function between groups either using ICS or placebo (244).

Summary: Airway inflammation is a key feature of asthma. Heterogeneity of the airway inflammatory cells has been described in adults with asthma (161). Although children with asthma and adults with asthma have some common features, they also differ in many other characteristics. Non-eosinophilic asthma is common and is less responsive to corticosteroid in adults. A study in adults with asthma found that clinical symptoms and lung function were similar across different airway phenotypes (161). The role of non-eosinophilic asthma in children is less clear. Also the relationship between airway phenotypes and clinical pattern in children with asthma is not described. An investigation of airway inflammatory phenotypes in children with asthma is necessary to understand the pathophysiology and treatment responses.
1.2-ASTHMA AND TOBACCO SMOKE EXPOSURE

Introduction

The harmful effects of active smoking on the human health have been well reported in many studies. Tobacco smoking is one of the major causes of morbidity and mortality in the worldwide.

In fact, not only active smoking but also passive smoking have affected human health. Environmental tobacco smoke contains the same toxic substances as identified in mainstream tobacco smoke. The adverse effects of passive smoking on the respiratory system have been demonstrated from infancy (245, 246) from children (247, 248) to adults (249, 250). Overall, there are increased incidences of respiratory illness and diminished pulmonary function in the children whose parents smoke. Mothers who smoke or are exposed to ETS during pregnancy cause an impairment upon lung function of their newborn infants and their children (94, 251). Children who are exposed to ETS have significantly more respiratory symptoms and diseases such as chronic cough, phlegm, and asthma (95, 248).

Tobacco smoke

Epidemiology

According to the World Health Organization, there are approximately 1.25 billion smokers all over the world, with two-thirds living in the developing countries (252). A survey in Australia in 2002 reported that at least 20% Australian women smoked during pregnancy (253). The reported prevalence of ETS exposure in children varies between
studies, with high rates of tobacco smoke-exposed children reported if parental smoking prevalence is high (254). 38% of Australian children under 12 years live in homes where at least one adult is a regular smoker (255).

Epidemiological studies have found that the prevalence of childhood asthma is higher among children who live with smoking parents, more so when both parents are smokers, in comparison with those living with non-smoking parents (256, 257). Smoking in mothers is more strongly related to the development of asthma in their children than others who smoked (258, 259). Maternal smoking during pregnancy increases the prevalence of physician-diagnosed asthma and wheezing during childhood (260, 261), whereas postnatal ETS exposure in children is associated with the respiratory symptoms of asthma (259). In utero exposure to tobacco smoking is suggested to have an even stronger effect on the onset of asthma in children than postnatal ETS exposure (262, 263).

One study found that adolescents with asthma were more likely to have smoking parents and that smoking parents are likely to recruit their children to become smokers (264). Surprisingly the prevalence of parental smoking is significantly higher in children with asthma compared to in healthy children (265).

Children with asthma living with smoking parents may have decreased scheduled GP visits or health care contacts for asthma, which may result in the children not receiving adequate asthma management (266). Furthermore, asthmatic adolescents who smoke use less asthma medication when having asthma symptoms compared to asthmatic adolescents without smoking (267).
Assessment of tobacco smoke exposure

Components of tobacco smoke are present in inhaled smoke and can be detected in both active and passive smokers. The markers which are often used to indicate smoking status included exhaled CO, nicotine and cotinine in saliva, hair, urine, and blood (268, 269).

Carbon monoxide (CO)
Carbon monoxide is the major toxic pollutant which has been found in ETS (270, 271). CO is a colorless, odorless gas which is emitted by incomplete tobacco combustion (272). Following inhalation, CO competes successfully with oxygen in the bloodstream to form carboxyhemoglobin (COHb). Approximately 80% of the CO formed from heme degradation is exhaled (273). In this form, CO has a short half life, about 5-6 hours (274).

Exhaled CO levels in smokers are significant higher than in nonsmokers (274, 275). There are significant positive correlations between exhaled CO levels and daily cigarette consumption, as well as duration of smoking in healthy smokers (275-277). Exhaled CO has been suggested as indication of active smoking habit (274). High-expired CO levels indicate current smoking. A CO level of 6 ppm was used as the cutoff in order to distinguish between smokers and non smokers, with a specificity of 98% and a sensitivity of 79% (274). Another study by Jarvis chose the optimal cutoff as 8ppb, giving 90% sensitivity and 89% specificity (278). Many studies use 10ppm as the cutoff to detect smokers (279, 280).
Carbon monoxide in expired air has also been accepted as an indirect measurement for passive smoking (281). Passive smoking is the major contributor to increased exhaled CO in healthy nonsmokers (274, 276, 282). Exhaled CO levels increased 1.4-2.7 times compared to the background exhaled CO levels after 30 minutes of ETS exposure, but only slightly increased after 10 minutes of ETS exposure (282). Significant relationships were found between the number of smoked cigarettes daily in the houses and the increase in exhaled CO levels in both healthy children and children with asthma who were non-smokers (281). Exhaled CO levels in non-smoking adolescents doubled if their mothers smoked indoor (283).

Nicotine and Cotinine levels
Nicotine has been seen as a biomarker of cigarette smoke (284). Measurement of nicotine can be performed to assess both active and passive smoking (285, 286). However, nicotine has a very short half life in body fluids, about 2 hours in the blood, then it is metabolized and excreted in the urine, so that nicotine measurement in the body fluid may represent recent tobacco exposure (287). A significant correlation was found between the number of cigarettes smoked and hair measurements of nicotine ($r = 0.48, p = 0.004$) in smokers (277). Recently, hair nicotine measurement may be a potentially useful, noninvasive technique to investigate ETS exposure (288). Hair nicotine levels may differentiate between children who were reported to be exposed to ETS and non-ETS exposed children, with higher hair nicotine levels among ETS exposed children (269, 289). The levels of nicotine in children’s hair were positively related to the number
of cigarettes smoked in the house by all household adults and the number of smokers in the houses (290), especially smoking mothers (291).

Cotinine is one of major metabolites of nicotine and may reflect the degree of ETS exposure (250, 286). Compared with nicotine, cotinine has a longer half life, ranging 1-2 days (7-40h) among nonsmoker adults who are exposed to ETS, 32-82 h in children, and even up to 160 h in neonates (287, 292). Cotinine values are not only used to distinguish smokers from nonsmokers but also to evaluate passive smokers. Cotinine can be measured in blood, hair, saliva and urine. An experimental study in children (4-11 years) who were been exposed to ETS at an air nicotine level of 110 mg/m3 for 2 hours in a bus indicated that urinary cotinine levels increased rapidly to a maximum after a median of 6 hours from the end of exposure; then remained at an apparent plateau for 12 hours; and decreased exponentially (293). Cotinine levels in infants are higher than in older children or adults despite the same reported amount of ETS exposure (294). A difference in the urinary elimination half-life of cotinine between infants and older children is suggested to cause the different cotinine levels (294).

Differentiation between smokers and non-smokers has been observed for all the different samples in terms of cotinine (295-297). Serum cotinine levels of 15 ng/mL was estimated a cutoff point to distinguish non smokers from smokers, with the best combined levels of sensitivity (95%) and specificity (96%) (298). Increased serum cotinine levels are significantly associated with the number of smokers in the households and ETS exposure (299). Salivary cotinine levels in children are heavily dependent upon to parental smoking (300). Urinary cotinine levels in the children are also significantly correlated
with the number of cigarettes smoked by parents, especially mothers who smoked (301, 302). Investigation ETS exposure in newborns have found that if the infants living with both other people and the mothers who smoked, neonatal hair levels of nicotine and cotinine were threefold higher than those living with others who smoked, but not mothers (303).

In summary, nicotine and cotinine levels in urine samples of non-smoking children living with smoking parents are significantly higher than of children without parental smokers. Moreover, maternal smoking shows stronger detectable biomarkers in their children than parental smoking.

**Active smoking and asthma**

An interaction between cigarette smoke and asthma has been described, with increased asthma morbidity and mortality, a change in airway inflammatory pattern, and relative glucocorticoid resistance, occurring in active smokers with asthma.

**Active smoking and asthma development**

The relationship between tobacco smoke and asthma is well described. Active cigarette smoking is a risk factor for the development of asthma in adults (250, 304). The prevalence of current smoking in asthmatic subjects differs in studies, ranging from 17% to 35% (305-307). A study of 1002 pregnant women in Japan has shown that active smoking was independently related to an increased prevalence of asthma after the age of 18 years (OR: 2.66; 95% CI: 1.30-5.38) (308). On the other hand, the prevalence of subjects with asthma who smoke is high. An investigation at 64 emergency departments
in 21 US states and 4 Canadian provinces found that 35% of the enrolled asthmatic patients were current smokers whereas 23% were former smokers (307).

In fact, different phenotypes of asthma may be associated with smoking. Studies have demonstrated that cigarette smoking is a risk to the onset of asthma in non-atopic subjects (309) whereas asthma in subjects who have asthma before smoking or are exposed to ETS is strongly associated with atopy (310).

**Effects of active smoking on the clinical symptoms of asthma**

Smoking has been suggested as a cause to increase asthma symptoms in smokers with asthma (305, 311). Current smoking is associated with an increase in emergency department visits and increased hospital care requirements for asthma (307, 312). Compared with nonsmokers with asthma, active smokers have more severe asthma symptoms, and faster decline in lung function (305, 313). Adult smokers with asthma are likely to develop irreversible airway obstruction resulting in chronic obstructive pulmonary disease (92).
Figure 1.2.1: Interactions between asthma and cigarette smoking

FEV$_1$: forced expiratory volume in one second; †: increase; ‡: decrease. Thomson at al, *Eur Respir J* 2004 (314).

Because of poor asthma symptom control, smokers with asthma need more reliever medication compared to non smokers with asthma (315). In addition, there was no improvement in lung function and AHR in smokers with asthma after three weeks of high dose inhaled corticosteroid (316).

In a study of 220 smokers who were diagnosed with asthma, subjects were divided in three groups: smoking cessation, smoking reduction and continuation of usual smoking. After 4 months follow up, improvements in asthma symptoms were observed in the smoking cessation group, including improvement in quality of life scores, a reduction in asthma medication requirements such as short acting β$_2$ – agonists and ICS doses, a
decrease in asthma symptoms and improvement in AHR. In the smoking reduction group, there were smaller improvements in clinical symptoms such as reduction of night use of $\beta_2$ – agonists, ICS doses, and reduced bronchial hyperreactivity whereas no changes in asthma symptoms occurred in the continuous smoking group (314, 317).

Overall, there is a strong association between active smoking and asthma. Smoking is a risk for asthma development, and asthmatic subjects who smoke have more severe disease, lower quality of life, and increased asthma medication use.

**Passive smoking with asthma**

Passive smoking or exposure to ETS or exposure to secondhand smoke is defined as a nonsmoking person is exposed to tobacco combustion products by others who smoke (285).

The interaction between active cigarette smoke and asthma has been well described. But whether passive smoking has the same role as active smoking on a person’s health is less clear. In fact, ETS exposure can occur in both adults and children, and may be especially dangerous if subjects have asthma. ETS has been reported to cause the development of asthma as well as to trigger asthma attacks (250, 318).

*Passive smoking in adults*

Passive smoking is attributed to the development of asthma in adults. ETS exposure is suggested as a cause for new-onset asthma and asthma exacerbation among adults (319).
Passive smoking in the workplace was significantly related with all types of respiratory symptoms and current asthma (OR: 1.90, 95% CI: 0.90 to 2.88) (320).

ETS exposure also relates to the severity of asthma. Among women with asthma, those with the highest serum cotinine levels had decreased lung function, including reductions of FEV\(_1\), FVC, and FEV\(_1\)/FVC ratio (321). Moreover, ETS exposure induced the increased bronchial responsiveness in subjects with asthma (320, 322).

### Passive smoking in children

#### Development of childhood asthma

Numerous studies have identified the significant relationship between parental smoking and development of asthma in children (95, 323). Epidemiologic studies have reported that children who live in the homes of cigarette smokers have a higher risk of asthma development than children who live in the homes of non-smokers (324). An increased prevalence of asthma in children of smoking mothers has been well reported (325, 326).

Children who are exposed to ETS have a higher risk for the development of asthma than children without ETS exposure (257, 327). The odds ratio for having childhood asthma in children exposed to ETS was 1.78 (95% CI: 1.33-2.31) in comparison with non ETS exposed children (328). If children with ETS exposure of more than 8 h per day, the risk for asthma development was significantly increased (OR: 2.06; 95% CI: 1.07 - 3.97), and the prevalence of wheezing was elevated (OR: 2.12; 95% CI, 1.25 - 3.58) (329).
The fetus can be exposed to ETS either by the mother who smokes or by her exposure to ETS by others during pregnancy. Studies have found that maternal smoking during pregnancy may be independently responsible for early onset asthma (92, 330). In utero exposure to maternal smoking without subsequent postnatal ETS exposure was associated with an increased prevalence of physician-diagnosed asthma (OR: 1.8; 95% CI: 1.1 to 2.9) (260). Children whose mothers smoked during pregnancy had an elevated risk of asthma in the first 5 years of life (OR: 1.6; 95% CI: 1.0 - 2.6)(261). The children of non-smoking mothers who were exposed to ETS throughout the pregnancy had an increased risk of childhood asthma diagnosis in the first 5 years of life (OR: 1.5; 95% CI: 1.0 -2.3), and for persistent asthma (OR: 1.5; 95% CI: 1.0 - 2.3) (261).

A prospective cohort study found that if mothers smoked four cigarettes daily, children had a 14% increase in the prevalence of wheeze, but the prevalence of childhood wheeze by age 10 had a 49% increased if mothers smoked 15 cigarettes or more daily when compared with children of nonsmoking mothers (331). In contrast, no increased risk was found in children of mothers who stopped smoking prior to the pregnancy (OR: 0.9; 95% CI: 0.5 to 1.5) (261).

Passive smoking may be a particular risk factor for the development of asthma in atopic children. Cantani found that IgE levels were higher and atopic symptoms started earlier in infants of smoking atopic parents (256). However, other studies have suggested that maternal smoking is a significant risk factor for the development of asthma in a subgroup of children with negative skin prick testing (100, 332).
Effects of passive smoking on the clinical symptoms of asthma

Although the correlation between passive smoking and asthma has been described, the question of whether passive smoking can induce asthma exacerbations is not clear. Studies have found that ETS exposure is commonly associated with increased asthma symptoms (133) episodes of respiratory infections (333), exacerbations and frequent hospitalization of children with asthma (250).

Both in utero exposure to maternal smoking and ETS exposure to infants have been associated with persistent deficits in lung function in both children with asthma and healthy children (334, 335). Children are more likely to wheeze if their mothers smoke (256, 260, 333). Another study reported an association between high levels of ETS exposure and increased nocturnal symptoms in childhood asthma (OR: 2.83; 95% CI: 1.22-6.55) (336). In addition, smoking in the home by people other than parents was significantly associated with asthma symptoms as well as a history of asthma (134). Interestingly, female adolescents whose mothers had smoked heavily during pregnancy and in the early months of their lives may have increased asthma symptoms in adolescence (262). Increased asthma morbidity, poor asthma control and lower quality of life have been reported in asthmatic children with ETS exposure (334).

There is also association between raised urine cotinine levels and increased asthma exacerbations of among children (257, 337). A survey in US in 1993 reported that ETS exposure exacerbated the symptoms of 20% of children with asthma (338). Children who have asthma and whose parents smoked have more frequent asthma attacks and more
severe symptoms (334). There was significant correlation between hospital admission and ETS exposure in children with asthma (OR: 6.63; 95% CI: 2.5-17.8; p = 0.002) (339).

The recovered period of childhood asthma after hospitalization for asthma attacks is impaired by exposure to ETS, characterized by persistent respiratory symptoms and frequent use of relief medication (340).

### Inflammation in smokers with asthma

**Circulating peripheral blood cells**

Asthma is known to be associated with raised circulating eosinophils (341). However, circulating eosinophil counts are decreased in smokers with asthma compared with non-smoker with asthma (342). This study also found that the proportion of blood eosinophils increases again if subjects stop smoking. This demonstrates that smoking has the potential to modify the inflammatory response in asthma. The reasons for the decrease in circulating eosinophils in blood in smokers with asthma are currently unknown. However, the alteration in circulating eosinophils raises the question of whether inflammation of the airways in smokers with asthma differs from non-smokers with asthma.

**Airway inflammation in smokers with asthma**

A fundamental question is whether smoking induces a distinct airway inflammatory phenotype of asthma compared to non-smoking. Cigarette smoking induces airway inflammation in normal smokers, with elevated neutrophil numbers (343), T-
lymphocytes (mainly CD8) (344), and macrophage numbers (343). There are few data on the potential influence of smoking on asthmatic airway inflammation. Cigarette smoking may modify airway inflammation in subjects with asthma, with the predominance of a non-eosinophilic inflammation (311). Experimental studies in animal models have shown that cigarette smoking may induce neurogenic inflammation (345), elevated oxidative stress (346), which leads to an increase in cysteinyl leukotrienes, and an amplification of airway inflammation (347). Reduced sputum eosinophils and elevated sputum neutrophils have been observed in asthmatic smokers (348). In summary, active smoking alters airway inflammatory cells in subjects with asthma, which is manifest as neutrophilic airway inflammation, including increased neutrophils and/or reduced eosinophils (314). The mechanism of the difference in airway inflammation in asthmatics smokers compared to asthmatic non-smokers is not clear. The reduced sputum eosinophils in smokers with asthma may be explained by an increase in eosinophil apoptosis caused by toxic components in tobacco smoke (349). Some substances derived from cigarette smoke such as CO may also attenuate airway eosinophil influx (350).

Cytokines and mediators

Sputum IL-8 levels are elevated in smokers with asthma. A higher release of IL-8 by LPS stimulated blood cells after smoking is observed, which may explain an increased neutrophil chemotaxis (351). Sputum IL-8 levels are significant correlated with sputum neutrophils and the history of smoking and negatively correlated to FEV₁% predicted (348). IL-8 is the main neutrophil chemoattractant in the lung, and smoking induced changes in IL-8 may explain the airway neutrophilia in smokers.
Cigarette smokers have been observed to have an increase in IL-2 (352), IL-4 (353), and TNF-α (354, 355) compared to non-smokers. In contrast, IL-10 was decreased in smokers (356). Imbalance in the TNF-α / IL-10 ratio may contribute to corticosteroid resistance in asthma (314).

IL-18 levels in the sputum supernatant of both normal subjects and subjects with asthma are reduced in smokers compared with non-smokers, with the lowest reduction reported in smokers with asthma. Similarly, IL-18 mRNA expression is decreased in smokers with asthma compared to non-smokers. IL-18 is a cytokine, which plays a role in the development of Th1 lymphocyte responses, and may regulate Th2 lymphocyte responses. The reduction of IL-18 in the smokers with asthma suggests that cigarette smoking may modify airway inflammation though changing the balance of Th1/Th2 cytokine secretion (357).

Several other mediator changes have been observed in smokers with asthma. Lipocortin-1, an anti-inflammatory protein of respiratory tract is increased in BAL fluid in smokers with asthma compared with non-smokers with asthma (358). Leukotriene B₄ release from peripheral blood lymphocytes is elevated in asthmatic smokers compared to asthmatic non-smokers (359).

Cigarette smoking may decrease FeNO level in both healthy subjects and subjects with asthma (360-362). Cigarette smoking can inhibit iNOS, which results in reduced FeNO levels. The effects of cigarette smoking on iNOS may be explained by the feedback
inhibitory mechanism because high levels of exogenous NO release by smoking (363), or
the carbon monoxide in the cigarette smoke that contributes to inhibition of iNOS (364).

**Airway inflammation in subjects with asthma who are exposed to ETS**

Not only active smoking, but passive smoking may potentially alter the airway inflammatory phenotype. Currently, no publications report the effects of passive smoking on airway inflammation in humans. A study in mice who had been exposed to ETS over the short term found reduced BAL eosinophil numbers in comparison with mice without ETS exposure (365). An experimental study also found that normal subjects had decreased FeNO levels after inhalation of passive smoke (366). Warke *et al* have reported an association between parental smoking and lower FeNO levels in children with asthma (367). However, others showed no difference in FeNO levels in childhood asthma with or without ETS exposure (368, 369).

**Airway hyperresponsiveness**

Cigarette smoking enhanced AHR in elderly asthmatics (359). A double blind placebo – controlled study demonstrated that smokers with asthma fail to show an improvement in AHR after three-weeks of high dose inhaled corticosteroids (316).

The correlation between ETS exposure and AHR in children is unclear. An experimental study in mice indicated that exposure to ETS may induce AHR (324). Other studies in murine models found increased bronchial hyperreactivity following allergen challenge after ETS exposure compared to those without ETS exposure (370). A study in 4 week
old infants found that airway responsiveness to histamine was significantly higher in infants exposed to parental smoking compared to those without smoking parents (371).

In other studies, AHR in children was found to be unrelated to parental smoking (372). An investigation in 249 children with asthma aged 7-11 years have noted that children whose mothers smoked have a lower frequency of AHR compared to those whose mothers do not smoke (373).

A review have reported that a small but significant effect of ETS on AHR in school aged children exposed to maternal smoking (OR: 1.29; 95% CI: 1.1-1.5) (374), suggesting the effect of ETS exposure in healthy children. However, a study reported that maternal smoking only affected AHR in non-atopic children who were exposed to equal or more than 10 cigarettes per day (375). In subjects with asthma, sputum eosinophils are associated with the degree of AHR (178). Active smokers with asthma demonstrated altered airway inflammatory cells, including increased sputum neutrophils and reduced eosinophils (314). Reduced sputum eosinophils may partly explain less AHR in smokers with asthma compared to non-smokers with asthma. The findings may also possible explain lower frequency of AHR in children with asthma who were exposed to ETS.

**Airway remodelling**

Airway remodelling is a characteristic of asthma, but it becomes more severe in smokers with asthma, with an increase in the area of submucosal elastic fibres, which lead to change the airway structures (376). The presence of emphysema as consequence of the
airway structural changes in smokers with asthma is related with the smoking history (377). In ex-smokers with asthma who had a heavy smoking history, both residual volume (RV) and emphysema score were also increased when compared with never smokers with asthma (359). Currently, no studies report the association between passive smoking and airway remodelling in subjects with asthma.

**Treatment**

Inhaled corticosteroids are the most effective anti-inflammatory therapy for asthma in both adults and children (171). Little data are available on the impact of smoking on asthma treatment. Recent evidence has demonstrated that active smoking may be strongly related to a relative corticosteroid resistance in asthma for both short-term or long-term, and low dose or high dose treatment (313, 378, 379).

A study in patients with mild persistent asthma taking 400 μg of ICS daily indicated that there was a significant less improvement in the mean morning PEF (l/min) (161) and an increase in the number of asthma exacerbations (6 versus 1, respectively, p = 0.007) in smokers compared to non-smokers over a 12 week treatment period (380).

Active smoking also limits the efficacy of short-term ICS treatment in subjects with mild and steroid-naïve asthma (316). In a randomized placebo-controlled study, subjects with asthma received inhaled fluticasone propionate 1000 μg daily or placebo for 3 weeks. A comparison of asthma outcomes was made between smokers and non-smokers. Non-smokers with asthma had a significant increase in mean morning peak expiratory flow
(PEF), mean FEV₁, and geometric mean PC20, and a significant decrease in the sputum eosinophils following fluticasone compared to placebo. However, no significant changes were found in the smoking asthmatic patients for any of these parameters (316). Another study reported that although 81% subjects with asthma taking daily ICS according to international guideline, 43% has persistent daily respiratory symptoms, especially subjects who were current smokers or ex-smokers. The findings suggest that smoking inhibited the beneficial effects of ICS on asthma (381).

Active smoking not only impairs the efficacy of ICS, it also reduces the effects of short-term oral corticosteroids in stable asthma. A randomized, placebo-controlled, crossover study with 40 mg prednisolone per day or placebo for 2 weeks in smokers, ex-smokers and non-smokers with asthma was conducted. Non-smokers with asthma had significantly improved in FEV₁, morning PEF L/m, and asthma control score after oral prednisolone compared to placebo. However, no changes in asthma parameters following oral prednisolone were observed in smokers with asthma. Ex-smokers with asthma had a significant improvement in morning and night PEF but not in FEV₁ or asthma control score(382).

Smoking is known to influence on corticosteroid activity and metabolism. The mechanisms of corticosteroid resistance in smokers with asthma have reviewed by Thomson et al, with some potential pathways of cigarette smoke against the anti-inflammatory effects of corticosteroids (314).
1) *Corticosteroid pharmacokinetics:*

Cigarette smoking causes increased mucus secretion and increased airway mucosal permeability in both normal subjects (383) and smokers with asthma (384). The hypersecretion of mucus in airways of smokers with asthma compared to non-smokers with asthma may limit the access of ICS to glucocorticoid receptors (GRs) on target cells in the airways (314).

2) *Corticosteroid and β2-adrenergic receptor (β2AR) interactions:*

β2-agonists may have a role in the molecular mechanism of corticosteroid action by increasing the nuclear localization of GRs (385). Long-term cigarette smoking has been shown to decrease the density of β2 – adrenergic receptors on lymphocytes, and reduce the ligand binding to β2 – adrenergic receptors, resulting in downregulation of β2-adrenergic receptors in the smokers (386). In smokers with asthma, down-regulation of β2 – adrenergic receptor function can affect to the clinical response to β2 –agonist as well as the action of corticosteroid therapy (314).

3) *Inflammatory cell phenotype:*

Cigarette smoking modifies airway inflammatory cells in asthma. Decreased sputum eosinophil counts are observed in smokers with asthma compared to non smokers with asthma (316, 348). This finding suggests the reasons behind the poor response to corticosteroids in the smokers. Alternatively, increased sputum neutrophil counts are observed in smokers with asthma that may impair the effects of corticosteroid therapy (379).
4) Cytokine and inflammatory mediators:

The production of pro-inflammatory cytokines such as IL-4 (353), IL-8 (348), tumour necrosis factor (TNF-α) (355) have been reported to increase in subjects who smoke. The mechanisms of corticosteroid resistance by these cytokines are unknown. An experimental study observed that when T cells are individual stimulated with the combination of IL-2 and IL-4, a significant reduction in GRs-binding affinity is found, resulting in a less response to corticosteroids (387).

Studies in vitro have demonstrated that the developments of corticosteroid sensitivity or resistance might be implicated by the balance of TNF-α/IL-10 secretion during the course of an inflammatory disease (388). TNF-α causes on decreased GR concentration whereas IL-10 may enhance glucocorticoid action by increased GR concentration. In addition, while glucocorticoids and IL-10 inhibit nuclear factor- kB (NF-kB) activation, TNF-α activates NF-kB, a nuclear factor that relate to glucocorticoid resistance (389-391). IL-10 levels in sputum are decreased in the smokers compared to non-smokers (356). The combination of increased TNF-α and reduced IL-10 in smoker subjects may contribute to the mechanism of corticosteroid resistance in smokers with asthma.

The combustion of cigarette smoke releases many hazards, including NO. The high concentration of NO may reduce the ligand-binding affinity of GR (392).

5) Glucocorticoid receptor numbers or binding affinity:

Basically, there are two isoforms of human GR. GR-α, which is much more expressed than GR-β at both the mRNA and protein levels, binds hormone, translocates to the
nucleus, and regulates gene transcription, is downregulated by corticosteroids (393) whereas GR-β, which does not bind glucocorticoids (394), is mainly located in the nucleus and cannot transactivate glucocorticoid genes (314). The imbalance in GR-α / GR-β ratio may implicate the response to corticosteroids. The reduced expression of GR-α may impair corticosteroid therapy (393). Alternatively, overexpression of GR-β may inhibit the activity of the ligand-activated GR-α, which contributes to the mechanism for corticosteroid resistance (395, 396).

Cultured human bronchial epithelial cells from smokers found GRs with a lower binding affinity in comparison to non smokers (397). The findings suggest the lower binding affinity of GRs could contribute to corticoid resistance in smokers.

6) Pro-inflammatory transcription factor activation:

The expression of NF-kB is an indicator of cellular activation. NF-kB is associated with the activation of inflammatory cytokines, including TNF-α, IL-8 (388, 390). Cigarette smoke combustion can release many substances, including lipopolysaccharide (LPS), which is can induce NF-kB (398). NF-kB can suppress the GR-α function though phosphorylation of the GR and limit the binding of the GR to DNA (399). Another transcription factor, including activator protein-1 (AP-1), is overexpressed in the lungs of smokers (400). Subjects with asthma who have corticosteroid resistance demonstrate an exaggerated activation of AP-1 (391). The findings indicate the association between cigarette smoke and corticosteroid resistance in subjects with asthma.
7) **Corticosteroid cell-signalling system:**

Glucocorticoids, though recruitment of histone deacetylases (HDAC) have switched off inflammatory gene transcription (401, 402). Both HDAC1 and HDAC2 are localized in the airway cells, with intense staining in the epithelium and inflammatory cells. Smokers have reduced HDAC2 expression and activity in alveolar macrophages. Decreased HDAC, which may result in increased inflammatory gene expression following cell stimulation, impairs glucocorticoid actions in these cells (401).

There are currently no reports on the effects of passive smoking on the response to asthma treatment. There are studies that show clear adverse effects of active smoking on airway inflammation in asthma, and the responses to corticosteroid treatment. At present, children with asthma have significant exposure to ETS. There is an urgent need to understand if and how passive smoking may modify airway inflammation in childhood asthma, and to then extend these studies to investigate the influence of ETS exposure on treatment responses in children with asthma.
PART II- METHODS

Study subjects: Healthy children and children with asthma were recruited from the Outpatient clinics at John Hunter Children’s Hospital and by advertisement. All children were aged between 7-17 years. Children with asthma were invited to attend between one and three visits while healthy children attend at one visit. Children with asthma who were subjects of chapter I, chapter II and chapter III were the same cohort of children.

Study design: The studies used both cross-sectional and longitudinal designs. Both healthy children and children with asthma were required to perform fractional exhaled Nitric oxide measurements, exhaled breath condensate, spirometry, skin prick test, hypertonic saline challenge and sputum induction. Because hypertonic saline challenge
was performed at the initial visit, it was not possible to perform a post bronchodilator measurement of lung function.

2.1-CLINICAL METHODS

Questionnaires

Clinical asthma questionnaires

Parents of children with asthma and children were asked a series of questions to assess the stability of the child’s asthma in the preceding 4 weeks at each visit. The questions are given in Appendix II.

The child’s asthma symptoms and relevant history were surveyed using a questionnaire from a previous long term cohort study in the Hunter region (the RIFYL study) (178). Data collected included age of asthma diagnosis, history of wheezing and other respiratory symptoms, family history of asthma and allergic diseases, potential asthma triggers and asthma medication use (Appendix II).

Asthma Control Questionnaire

Asthma symptoms experienced over the previous week were assessed with the Juniper Asthma Control Questionnaire (ACQ) (Appendix III) (403). This is a 7-item questionnaire, which investigates asthma symptoms that physicians consider to be most important for defining of asthma control, the requirement of short-acting β2-agonist, and airway caliber (FEV₁). All questions are short and easy to complete. Analysis of this score determines the level of asthma control. In addition, the ACQ can discriminate
different levels of asthma control in each asthmatic subject. The ACQ is valid and recommended as the primary goal of asthma management as defined by international guidelines.

*Environmental Tobacco Smoke Exposure Questionnaires*

Smoking habits

Environmental tobacco smoke (ETS) exposure is thought to cause an increase in asthma symptoms and asthma exacerbations in children. Several valid questionnaires have been published to evaluate ETS exposure in subjects with asthma. Eisner et al have designed a series of questions to assess ETS exposure among adults with asthma (404). These included the numbers of smokers in the households, the numbers of cigarettes used daily, the numbers of visitors who smoked in the last week, the numbers of cigarettes that were used each visit, the time spent in public areas where other people smoked, the estimated ETS exposure, and the association between ETS exposure and asthma symptoms.

Previous studies have investigated ETS exposure in children, based on the number of smokers in households and the smoking habits of parents, including the places and times at which cigarette smoke was commonly used (405, 406). Berman et al used a survey to assess levels of household smoking and the associated ETS exposure by children with asthma (407). The study found an association between household nicotine levels and the children’s urinary cotinine levels. In addition, both household nicotine and children’s urinary cotinine levels were significantly correlated with the total number of cigarettes smoked per day in the home and total number of hours smoking indoors per week.
reported by the parents. Similarly, Irvine et al demonstrated that children with asthma whose parents smoked were exposed to high levels of ETS (300). Significant relationships were reported between salivary cotinine levels in children and the total number of cigarettes smoked daily, the amount smoked in the home daily, and the frequency of parental smoking in the same room as the child. Regular contact with smokers other than parents also led to similar ETS exposure in children.

In this study, we have modified the questionnaires from previous publications (300, 404, 407-410) in order to assess ETS exposure in children with asthma. These included: identification of who smoked in the household, the number of cigarettes smoked per day, where they smoked: inside or outside, estimated ETS exposure on children. These questions were shown in Appendix IV.

Knowledge and attitudes of parents towards ETS
ETS exposure in children, especially in children with asthma is still high. It is important to understand what features make the smokers continue smoking, or fail to prevent exposing their children to ETS. It has been suggested that the knowledge and attitudes of parents towards passive smoking may influence on their smoking habits (408). Also the nicotine addiction of smokers contributes to difficulty in attempts to give up cigarette smoking.

A cross-sectional study was performed in the five Nordic countries, with 5500 households that investigated the correlation between the health-risk awareness of parents
about the potential hazards of ETS for children and the levels of ETS exposure in children (408). This study reported that non-smokers were more likely to be aware of the health risk of ETS exposure in the children than smokers. Moreover, parental smoking may be a risk to recruit children to become smokers (411).

The dependence on nicotine is a crucial factor that the physicians should deal with if they want the smokers stop smoking. Nicotine addiction may prevent quitting despite fully understanding the adverse effects of tobacco smoke on their health as well as their children’s health. In 1990, the Fagerstrom Test for Nicotine Dependence (FTND) was designed to determine the levels of nicotine addition (409). The six questions with scoring for each item were simple, non-invasive, and easy to evaluate the nicotine dependence.

We assessed the knowledge and the attitudes of parents of children with asthma about passive smoking. Nicotine addiction was also assessed if the parent was a smoker. The questionnaires were modified from previous publications (407-409) and are given in Appendix V.

**Objective measurements**

*Nitric Oxide Measurement*

Nitric oxide is suggested as a mediator of airway inflammation. Measurements of Fractional exhaled Nitric Oxide (FeNO) were made according to American Thoracic Society (ATS) guidelines (412). Fractional eNO was measured by single breath online
technique, using a chemiluminescence NO analyzer (NIOX® Nitric Oxide Analyzer, Aerocrine AB, Solna, Sweden), expressed in parts per billion (ppb). The exhalation flow rate employed was kept to a constant 50 ml/s. Exhaled NO worksheet is given in Appendix VI a.

FeNO measurements were performed before spirometry because FeNO levels can be reduced after lung function measurements and sputum induction tests (413). Also patients were advised to fast about 2 hours before FeNO measurements as some foods and drinks have been found to increase exhaled NO levels(412).

Each child was required to perform at least three measurements. For each measurement, a child was asked to exhale for 10 seconds, and each measurement completed in this time period was called the NO plateau. The average FeNO level was calculated based on the three NO plateau levels (414).

Exhaled carbon monoxide measurement

There is a direct link between the smoking status of subject and the concentration of carboxyhaemoglobin (COHb) in the blood of subject, and the concentration of COHb is often estimated from measurements of carbon monoxide in the exhaled air (415). Carbon monoxide (CO) in expired air has been accepted as an indirect measurement of tobacco smoke exposure (281). The exhaled CO measurement is a non-invasive, cheap, safe and portable method to assess tobacco smoke exposure (415). The exhaled CO worksheet is given in Appendix VI b.
Exhaled carbon monoxide was assessed using the piCO Smokerlyzer Breath CO monitor (Bedfont Scientific Ltd, Kent, ME1 3QX England) expressed in parts per million (ppm).

The subjects were asked to inhale and hold their breath for 15 seconds, and then exhale slowly and gently into the mouthpiece. Exhalation was continued as long as possible. The level of exhaled CO was recorded. A reading of level 4 or less indicates non-ETS exposure or/and nonsmokers. A reading between level 4 and level 10 shows the subject is not a smoker, but may be exposed to ETS. Level 11 indicates active smoking (279, 280).

**Exhaled breath condensate**

Exhaled breath analysis is a non-invasive method to study airway inflammation and lung oxidative stress (416, 417). The collection of exhaled breath condensate (EBC) is feasible, simple and safe in both adults and children (418, 419). The success rate of EBC collection for children equal or more than 4 years is high, up to 100% (418). This study used the EBC collection technique described by Formanek (420).

Each subject was asked to sit comfortably at the collection device (mouthpiece closest to subject) and wear a nose clip. The test was explained the subject and they were asked to mouth breath normally into the mouthpiece for 10 minutes and to swallow any saliva that may build up in the mouth. The condensate was then collected and transported to the laboratory for analysis of pH.
Allergy skin prick testing

The principle of skin testing is based on the reaction between an allergen and sensitized IgE antibody in the skin. Mast cells with sensitized IgE attached can be activated by allergen, showing the features of degranulation (421, 422). Histamine induces a weal and flare response, which is measured to assess allergen sensitization. The mediator that plays an important role in the allergic reaction is histamine (422, 423). The use of antihistamine drugs before skin prick testing can inhibit the skin test results. Therefore, antihistamines are withheld for 5 days before skin prick testing.

Four common allergens, *Dermatophagoides pteronyssinus* (Dpt) (strength 30,000 AU/mL), *Alternaria tenuis* (1:10 w/v), cockroach mix (American, German, 1:10 w/v) and mixed grass (Kentucky Bluegrass, Orchard Grass, Redtop, Timothy, Sweet Vernal grass, Meadow Fescue, Perennial Ryegrass, strength 10,000 BAU/mL) were tested in both healthy children and children with asthma (Hollister-Stier Laboratories LLC, Spokane, WA, USA). The positive control was histamine HCL (10mg/ml) and the negative control was a normal saline/glycerin solution (50% v/v glycerin).

The tests were read at 15 minutes, and the length of long axis and the length of its perpendicular of allergic weal size were recorded. The cumulative size was calculated based on the average of the length of long axis and the length of its perpendicular of weal size. A positive reaction was as the weal diameter was equal or larger than 3 mm. A negative reaction was as the weal diameter was less than 3 mm. Atopy was determined as
the presence of at least one positive skin reaction. The allergy skin test worksheet is given in Appendix VI c.

Measurement of lung function

Lung function was assessed in all children using a spirometer (KOKO, Spirometry, Pulmonary data Service Instrument, Inc, Louisville, USA) according to ATS guidelines (424). The spirometer was calibrated daily, prior to spirometer use. The spirometer was calibrated adjusting for room temperature, humidity and barometric pressure. Spirometry was performed before saline challenge and sputum induction and at the end of each sputum induction period. The predicted lung function values of Hibbert et al were used as reference values (425). All subjects had estimation of FEV$_1$ and FVC based on age, height, gender and race.

The procedure was explained and demonstrated to each child. The child stood up in front of the spirometer. Wearing a nose clip, the subject was asked to breathe to maximal inspiration, and then forcefully exhale out to residual volume for at least 6 seconds. At least three technically acceptable manoeuvres were performed. The best FEV$_1$ and FVC were recorded.

Saline challenge and sputum induction

Sputum induction has been used as a non-invasive method to investigate airway inflammation in subjects with asthma since the first report of the technique in 1992 (426). The technique is feasible (162, 199), safe (162, 427) and gives reproducible results in
most subjects $\geq 6$ years (426, 428). According to Gibson et al, the reported success rates in sputum collection after sputum induction in children ranged from 68% to 100% (428). Sputum can be safely obtained during asthma exacerbations (172) as well as stable asthma (427). The sputum collected is a mixture of saliva and lower airway secretions (429). In order to reduce the contribution of saliva, sputum portions were selected.

Hypertonic saline indirectly causes bronchoconstriction in subjects with asthma (429). As a result, the hypertonic saline challenge test is also recommended to measure AHR (430, 431). A combination of hypertonic saline challenge and sputum induction can be used to assess AHR and AI in a single test at the same time (178, 432). The combined challenge and induced sputum by hypertonic saline is well tolerated and safe in children (432).

In this study, a combined saline challenge and sputum induction were performed after spirometry (178). The saline challenge and sputum induction worksheet is given in Appendix VI d. The subject was required to withhold some asthma medications prior to their appointments (Table 2.1.1).
Table 2.1.1: Asthma medications were withheld prior to their appointments.

<table>
<thead>
<tr>
<th>6 hours</th>
<th>12 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventolin</td>
<td>Serevent</td>
</tr>
<tr>
<td>Respolin</td>
<td>Seretide</td>
</tr>
<tr>
<td>Airomir</td>
<td>Oxis</td>
</tr>
<tr>
<td>Asmol</td>
<td>Foradile</td>
</tr>
<tr>
<td>Bricanyl</td>
<td>Symbicort</td>
</tr>
<tr>
<td>Atrovent</td>
<td>Singulair</td>
</tr>
<tr>
<td>Intal</td>
<td>Spiriva</td>
</tr>
</tbody>
</table>

Combined Hypertonic Saline Challenge/Sputum Induction

Children were asked to rinse their mouth with water and were then instructed in sputum expectoration. The child then inhaled a 4.5% hypertonic saline solution through a mouthpiece and two-way non-rebreathing valve box (Hans Rudolph Inc, Kansas City, MO, USA), connected to a DeVilbiss 2000 ultrasonic nebuliser (Oregon, Pike, PA, USA). Each child was asked to do this for 30 seconds, 1 minute, 2 minutes, 4 minutes, 4 minutes, and 4 minutes. Spirometry was performed 1 minute after each dose and the child was then asked to produce a specimen of sputum in the sterile container. The test was stopped after completion of challenge or if a child or their parents requested the challenge to stop. The dose of hypertonic saline used was calculated by weighing the nebulizer cup and the tube before and after sputum induction. If the FEV\textsubscript{1} dropped below 15% of baseline, reliever medication (Salbutamol, 200 μg) was administered, and the test continued when the FEV\textsubscript{1} had returned to within 10% of the baseline value.
Subjects were defined as having airway hyperresponsiveness (AHR) if the FEV\textsubscript{1} fell by ≥ 15% of baseline after inhalation of a provocative dose of ≤ 16ml hypertonic saline solution (4.5%).

2.2-LABORATORY METHODS

*Sputum processing*

Sputum was collected in a sterile container during the bronchial provocation challenge and sputum induction. It was refrigerated at 4\textdegree{}C while awaiting transport. Sputum was processed within 2 hours of collection to preserve adequate cell viability. Sputum portions were selected from saliva and processed as described (178, 426).

Induced sputum was collected for two purposes:

100 μL sputum sample was processed by ultracentrifuge for the assessment of TNF-\(\alpha\).

The remaining sputum sample (at least 100μL) was processed using DTT to prepare a cell differential and supernatant for IL-8 assessment.

All sputum samples were handled in a Class II biohazard cabinet. The sample jar was inverted into a clean open Petri dish, and was inspected against a black background. Clean forceps were used to try and separate out mucus clumps from saliva bubbles. All suitable mucus clumps were pipetted (minimum of 100 μL and not more than 1 mL) into labeled 15 mL tube using a positive displacement pipette. The actual volume of mucus
used was recorded on the work sheet. Four times the volume of 0.1% dithiothreitol (DTT; Sputolysin 10%) was added to the sample in the tube, and then mixed up and down with disposable pipette. The tube was capped and placed at room temperature for 30 minutes to dissolve mucus.

When 30 minute incubation period was over, the same volume of PBS as Sputolysin (i.e. 4 times the volume of the sputum used) was added; then was mixed with a disposable pipette and shaken for a further 5 minutes. The sputum and Sputolysin mixture was filtered through the nylon filter apparatus (Millipore; North Ryde, New South Wales, Australia), wet filter with PBS prior to filtering sample to ensure no loss of sample during filtering.

The post-filtered volume was recorded on the data sheet (to be used for total cell count). An autopipette was used to put 25 μL of Trypan Blue into an Eppendorf tube, then 25 μL of sputum sample was added, this was mixed well by slowly drawing up sample into pipette and then releasing back into Eppendorf tube. Those were repeated several times to ensure that the sputum sample and Trypan blue were mixed thoroughly. A light microscope was used to perform a Total Cell Count (TCC). The remaining cell suspension was centrifuged at 400 x g for 10 minutes and the supernatant was evenly aspirated into Eppendorf tubes labeled with a laboratory number and stored at –80 C. The pellet was resuspended in a volume of PBS to a concentration of 1 x 10^6 cells/mL.

Then 70 μL of the sample was added to a cytospin sample bucket, and sample was centrifuged (500 rpm for 5 minutes). The cytopsins were carefully and quickly removed
from apparatus; making sure to flip off the filter paper. Cytocentrifuge slides were air dried. The slide was labeled with a lab number.

Two slides were stained with either Chromatrope 2R (C2R), to detect eosinophils or May Grunwald Giemsa (MGG) for a general differential.

Cell counting

Cells were counted and identified using a 40x light microscope. A total of 400 cells were counted per slide. The characterized of cells were determined by their morphology which were described later. Squamous cells were identified and expressed as a percent of the total number of cells counted. The remaining cells were identified by their morphology and expressed as a percent of the total non-squamous cells counted.

Sputum quality was investigated based on the amount of squamous contamination, debris, cell outline, nuclear morphology, the presence of macrophages, and the number of cells on slice. The overall impression of slide was determined and scored.

*Cell quality assessment used for sputum cytospins*

Cytospin Quality

*Debris*: 4 (nil), 3 (scant), 2 (moderate), 1 (excessive).

*Cell outline*: 4 (preserved), 3 (isolated cell damage), 2 (many cells damaged), 1 (most cells damaged)

*Nuclear morphology*: 4 (preserved), 3 (isolated nuclei damage), 2 (many nuclei damaged), 1 (most nuclei damaged)
**Squamous:** 4 (20%), 3 (21-60%), 2 (61-85%), 1 (>85%)

**Overall impression:** 4 (good), 3 (acceptable), 2 (just acceptable), 1 (bad)

**Slide macrophages present:** 1 =yes  0=no

**Number of cells on slide:** 0=< 200;  1 = 200-399;  2 ≥ 400

**Table 2.1: Cell morphology**

<table>
<thead>
<tr>
<th></th>
<th>Cell size and shape</th>
<th>Nucleus size and shape and chromatin</th>
<th>Cytoplasm colour/ granules</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchial epithelial cell</td>
<td>10-20 μm oval or columnar</td>
<td>8 μm single, round purple, loose chromatin pattern</td>
<td>Blue-light purple</td>
<td>Cilia</td>
</tr>
<tr>
<td>Squamous epithelial cell</td>
<td>40-60 μm polygonal</td>
<td>5-10 μm single, round, purple, loose</td>
<td>Blue to pink</td>
<td></td>
</tr>
<tr>
<td>Macrophage</td>
<td>20-40 μm oval</td>
<td>12-15 μm round 1 or more purple dense</td>
<td>Blue/grey with foamy white vacuoles</td>
<td>Smokers have black/ purple dots</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>16 μm oval</td>
<td>3-5 lobes purple, tight</td>
<td>Pink/blue granules with blue cytoplasm</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>9-12 μm oval</td>
<td>1 round purple fills cell, tight</td>
<td>Sky blue thin rim</td>
<td></td>
</tr>
<tr>
<td>Eosinophil</td>
<td>16 μm</td>
<td>2-4 lobes purple dense</td>
<td>Brick red granules and pink cytoplasm</td>
<td></td>
</tr>
</tbody>
</table>
Measurement of interleukin-8 in sputum supernatant

IL-8 was assayed using the Human DuoSet ELISA Kit (IL-8 ELISA; R & D Systems, Inc. Minneapolis MN, USA) according to the manufacturer’s instructions, with a standard curve range from 31.2pg/mL to 2000pg/mL. This assay has a sensitivity of 5.6pg/mL. This assay has been previously validated (433).

Measurement of TNF-α in ultracentrifuge sputum sample

Human Tumor Necrosis Factor alpha (TNF-α) was assayed by the sandwich ELISA using the DuoSet ELISA Kit for TNF-α Cat no: DY 210 (R & D Systems, Inc. Minneapolis MN, USA) with a standard curve range from 31.3pg/mL to 1000pg/mL. The sensitivity of the TNF-α assay was 6.8pg/mL. Due to the effect of DTT on TNF-α (434), this cytokine was assayed using ultracentrifuge processed sputum samples.

Measurement of pH of EBC

The pH was measured after EBC collection using a pH meter (Shindengen, ISFET pH meter KS 723, Tokyo, Japan) without deaeration (435, 436). Although degassing with argon is a recommendation for some of EBC exponents, but not a scientific prerequisite.

Measurement of cotinine in urine

Children’s urine was collected for measuring urinary cotinine using Nic-Alert test strips (Nymox Pharmaceutical Corporation, Maywood, NJ). NicAlert tests were used to identify active smoking as well as to detect passive smoking exposure. The tests can
provide quick, accurate determination of an individual’s smoking status and the levels of ETS exposure (437, 438).

Table 2.2.2: Results and interpretations

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration (ng/ml)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0-10</td>
<td>Non-user of tobacco products.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimal to no exposure.</td>
</tr>
<tr>
<td>1</td>
<td>10-30</td>
<td>Non-user of tobacco products.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low passive nicotine exposure.</td>
</tr>
<tr>
<td>2</td>
<td>30-100</td>
<td>Non-user of tobacco products.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higher passive nicotine exposure.</td>
</tr>
<tr>
<td>3</td>
<td>100-200</td>
<td>User of tobacco products.</td>
</tr>
<tr>
<td>4</td>
<td>200-500</td>
<td>User of tobacco products.</td>
</tr>
<tr>
<td>5</td>
<td>500-1000</td>
<td>User of tobacco products.</td>
</tr>
<tr>
<td>6</td>
<td>1000+</td>
<td>User of tobacco products</td>
</tr>
</tbody>
</table>
2.3-DATA MANAGEMENT AND ANALYSIS

Statistical methods
Statistical analysis was carried out using STATA version 9 (STATA Corporation, College Station, TX, USA).

Continuous variables that were approximately normally distributed were summarized with means and standard deviations and non-normal data with medians and inter-quartile range. The frequency distribution of discrete data is presented as percentages.

A comparison of two groups
The correlation between the outcome variables and other variables was investigated. Methods of examining the relationship between two continuous variables depend upon the distribution of the variables. For variables with normal distribution, Student’s t test was carried out to compare differences between two groups. If the data was severely skewed, there were two approaches that could be taken. Firstly, the data may be evaluated by log transformation. Alternatively if this approach did not produce a normal distribution, a non-parametric method was carried out. To detect differences between two samples, which came from independent populations the Mann-Whiney test was used.

A comparison of multiple variables
One-way ANOVA was applied to compare the differences in more than 2 independent variables. STATA can carry out multiple comparisons using the Bonferroni, Sidak and Scheffe procedures. However, the test was only conducted in case of a normally
distributed population. The Kruskal-Wallis test may be used to test for differences between the central tendencies of three or more groups when assumptions for the one-way ANOVA did not hold.

Comparison of categorical variables
The Chi-square method was used to test hypotheses about categorical data summarized in a contingency table, comparing 2 or more variables. The Chi-square test was required that the sample size was large enough so that the Chi-squared approximation was appropriate, and no cell should have an expected count less than 5. If the sample size was such that the Chi-squared approximation was not appropriate, Fisher’s exact test was used.

The association between two categorical variables was expressed by odds ratio and 95% confidence intervals. The sensitivity, specificity and Youden’s index of clinical assessments were calculated in order to estimate the cut-point for prediction of inflammatory phenotypes in children with asthma.

Correlation
Correlation was used to measure the strength of the relationship between the variables. If the data was normally distributed, Pearson’s correlation coefficient was conducted. In case of non-normal distribution, associations between continuous variables were examined using Spearman’s rank correlation coefficient (r).

A p < 0.05 was accepted as statistically significant.
Data entry and management

Each subject’s data was recorded using a confidential case report form. Prior to data entry, all worksheets were checked to look for missing data and to make sure the data collected was correct. All data collected were filled in on hard copy and then securely stored.

Data was entered into databases designed specifically for this study using Microsoft® access (Microsoft Corporation, USA). Data entry was also checked independently after completion.

2.4- ETHICAL CONSIDERATIONS

The research project was undertaken as part of this thesis. The study was approved by the University of Newcastle’s Human Research Ethics Committee and the Hunter Area Research Ethics Committee. The study in Hanoi (Vietnam) was approved by the Director of BACHMAI hospital.

In the research involving human subjects, a balance between the interventions on the subject’s health and the achieved values by conducting the studies should be carefully considered. In order to conduct studies in humans, studies should be approved by the Research Ethics Committee which can ensure that:
1- Voluntary participation will be respected by written informed consent, and there is a cooling off period between invitation, consent and commencement in the study. The study should separate the treating physician from the consent process. If subjects agree to participate but decide at a later date to withdraw, they are free to do so. Withdrawal will not affect the subject's current or future management.

2- Participants have their integrity, autonomy and privacy respected. The participant's identity, medical records and data collected relating to the study will remain confidential information. Participants will be identified for the purposes of the study by initials and an assigned participant trial number. All data collected for the purposes of the study will be transcribed into a separate folder in addition to the medical records, and participants will not be identifiable from these folders. Data will be securely stored and in confidence. Only authorised staff working on the study will have access to the data and information collected during the study.

3- Any physical harm and risks of harm for participants from the studies should be minimized. The risks of harm must be informed in the information sheets for participants to recognize.
PART III – RESULTS

CHAPTER I

COMPARISON IN AIRWAY INFLAMMATORY MARKERS BETWEEN
HEALTHY CHILDREN AND CHILDREN WITH ASTHMA

Introduction

Asthma is a common respiratory disease, especially in English speaking countries such as Australia. The prevalence of asthma symptoms in children varies from country to country. The diagnosis of asthma in children (especially aged less than 5 years) is based upon the clinical symptoms (156). However, asthma is a complex disorder, which cannot be defined solely in terms of clinical pattern.

The most recent definition states that asthma is a chronic inflammatory disorder of the airways in which many cells play a role and the inflammation causes an associated increase in airway responsiveness to a variety of stimuli (439). This then results in variable airflow obstruction and symptoms. Airway inflammation is suggested as a key feature of asthma.

Currently, airway inflammation can be detected through both invasive and non-invasive methods. Non invasive methods such as exhaled Nitric oxide (368, 440), exhaled breath condensate (418), and sputum induction (426, 428) are safe, feasible and potentially useful to assess airway inflammatory markers in both healthy children and children with
asthma. There has been little study of how these markers perform in comparison to each other in the assessment of airway inflammation.

**Aim**

The aim of this study was to investigate and compare the clinical symptoms and airway markers between healthy children and children with asthma. The parameters that were assessed included lung function, atopic status, AHR, exhaled NO, pH of EBC and induced sputum cell counts. The research sought to distinguish airway inflammation in children with asthma compared with healthy children.

**Hypotheses**

We hypothesized that children with asthma have increased airway inflammatory markers compared with healthy children.

Further, we hypothesized that induced sputum analysis would show a difference in sputum cell counts between healthy children and children with asthma, that exhaled breath condensate pH in children with asthma would be lower compared with healthy children, and that FeNO levels in children with asthma would be increased in comparison with healthy children.
Methods

Study design

This study used a cross-sectional analytical design. Healthy children and children with asthma were recruited from the Outpatient clinics at John Hunter Children’s Hospital and by advertisement. All children were aged between 7-17 years. Information sheets were sent to parents of children with asthma, parents of healthy children, childhood asthma and healthy children. Two weeks after sending the letters, the parents were contacted by phone to invite them and their children to participate in the study. Written informed consent was obtained from those agreeing to participate.

Children with asthma were previously diagnosed by pediatricians based on clinical and lung function criteria. Each child’s asthma was then classified using the criteria from National Asthma Council Australia (156).

Both healthy children and children with asthma were required to perform FeNO measurements, exhaled breath condensate, spirometry, skin prick test, hypertonic saline challenge and sputum induction. Children with asthma were required to withhold short acting β2-agonists at least 6 hours and long acting- β2 agonists for 12 hours prior to their appointments.

Subjects

Children with stable asthma were defined as having no increase in asthmatic symptoms or asthma medication use, no treatment with oral corticosteroid, and no unscheduled visit to a GP or hospital due to worsening asthma in the preceding 4 weeks. Exclusion criteria
included an increase in asthmatic symptoms such as wheeze, shortness of breath or cough, or features of a lower respiratory tract infection, an increase in asthma medication use, treatment with oral corticosteroids, and an unscheduled visit to a GP or hospital due to an asthma attack in the preceding 4 weeks.

Healthy children were defined as having no diagnosis of any respiratory diseases, no diagnosis with other significant morbidity, and no treatment with antibiotics during the preceding 4 weeks. Exclusion criteria were defined as use of any asthma medication, diagnosis of any respiratory diseases, diagnosis with respiratory infection within the preceding 4 weeks, treatment with antibiotics during the preceding 4 weeks, or having any significant illness.

**Measurements**

**Questionnaires**

Parents of children with asthma and children were asked a series of questions to assess the stability of asthma in the preceding 4 weeks. Asthma control were assessed using the Juniper asthma control score (403).

**Nitric Oxide Measurement**

Measurements of fractional exhaled nitric oxide (FeNO) were made according to ATS guidelines (412), using a chemiluminescence NO analyzer. Each child was required to perform at least three measurements. The average FeNO level was calculated based on the three NO measured levels (414).
Exhaled breath condensate

Children were seated, wearing a nose clip, and asked to breathe normally with tidal breathing for 10 minutes into a mouthpiece attached to a cooling system. Children exhaled through a one-way valve and saliva trap to avoid saliva contamination. The Teflon condenser tube was wrapped in ice packs to keep the system cool through the procedure. As the children exhaled, the air was cooled and condensed (441). EBC samples were collected and then EBC pH was measured immediately, using a silicon chip sensor.

Skin prick testing

Allergen sensitization was determined by skin prick tests, which were performed on the forearm of the children with four common allergens, Dermatophagoides pteronyssinus (DP), Alternaria tenuis, cockroach mix and mixed grass. The positive control was histamine HCL (10mg/ml) and the negative control was a normal saline/glycerin solution (50% v/v glycerin). A positive reaction was defined as the weal diameter was equal or larger than 3 mm. Atopy was determined as the presence of at least one positive skin reaction.

Spirometry

Spirometry was performed using an electronic spirometer according to ATS guidelines (424). Children were asked to stand up and wear a nose clip during the test. After breathing to total lung capacity, children forcefully exhaled to residual volume. Each
child was required to perform three consistent tests. The best FEV$_1$ and FVC were recorded.

**Saline challenge and sputum induction**

In this study, combined saline challenge and sputum induction were performed after spirometry. The procedure of saline challenge and sputum induction has been described in Methods chapter.

**Sputum processing**

Sputum portions were selected from saliva and processed as described (178, 426). Cytospins were prepared from the resuspended cell pellet (Shandon Cytospin, Sewicke, PA, USA). Two slides were stained with either Chromatrope 2R (C2R), to detect eosinophils or May Grunwald Giemsa (MGG) for a general differential.

**IL-8 measurement**

IL-8 was assessed in sputum supernatant using the Human DuoSet ELISA Kit (IL-8 ELISA; R & D Systems, Inc. Minneapolis MN, USA) with a standard curve ranges from 31.2pg/mL to 2000pg/mL.

**TNF-α measurement**

Human TNF-α in ultracentrifuge sputum sample was assayed using the DuoSet ELISA Development Kit for TNF-α Cat no: DY 210 (R & D Systems, Inc. Minneapolis MN, USA) with a standard curve ranges from 31.3pg/mL to 1000pg/mL.
Statistical analysis

Statistical analysis was carried out using STATA (STATA Corporation, College Station, Texas, USA). Characteristics of the study population and parametric results were expressed as geometric mean and standard deviation (SD). Non-parametric results were reported as median and inter-quartile range (IQR). Group comparisons were conducted. For variables with normal distribution, Students t-test was performed. Nonparametric data was analyzed by the Mann-Whitney Test for two group comparison or log transformation for normal distribution. Chi- squared test was carried out for categorical data. If the sample size of each cell in the table was less than 5, Fisher’s exact test was used. The Spearman rank correlation was used to determine the association between non-parametric data. A p < 0.05 was accepted as statistically significant.
**Results**

*Subject characteristics*

A total of eighty-six children aged between 7 and 17 years were recruited for this study. There were twenty healthy children and sixty-six children with asthma.

*Table 3.1. 1: Demographic characteristics of healthy children and children with asthma*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Healthy control (n=20)</th>
<th>Asthma (n=66)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean (SD)</td>
<td>10.85± 3.5</td>
<td>11.1± 3.2</td>
<td>0.8*</td>
</tr>
<tr>
<td>Gender (%male)</td>
<td>30</td>
<td>54.5</td>
<td>0.06 #</td>
</tr>
<tr>
<td>Height, cm, mean (SD)</td>
<td>147.78 ± 16.8</td>
<td>147.85± 15.8</td>
<td>0.99*</td>
</tr>
<tr>
<td>Weight, kg, mean, (SD)</td>
<td>42 ± 16.9</td>
<td>46.5 ± 18.2</td>
<td>0.32*</td>
</tr>
<tr>
<td>BMI (Body Mass Index) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24.9</td>
<td>95</td>
<td>78.8</td>
<td>0.09 $</td>
</tr>
<tr>
<td>≥ 25</td>
<td>5</td>
<td>21.2</td>
<td></td>
</tr>
<tr>
<td>FEV₁% predicted, mean (SD)</td>
<td>98.85 ± 15.6</td>
<td>91.9 ± 13</td>
<td>0.05*</td>
</tr>
<tr>
<td>% FEV₁/FVC, mean (SD)</td>
<td>88.9 ± 8.9</td>
<td>81.6 ± 8.5</td>
<td>0.002*</td>
</tr>
<tr>
<td>AHR (%)</td>
<td>0</td>
<td>59.7</td>
<td></td>
</tr>
<tr>
<td>ICS used (%)</td>
<td>0</td>
<td>63.6</td>
<td></td>
</tr>
<tr>
<td>ICS dose, μg/daily median (IQR)</td>
<td></td>
<td>250 (0-800)</td>
<td></td>
</tr>
</tbody>
</table>
There were no significant differences in age, height, weight, and gender between the healthy children compared to the children with asthma. However, the prevalence of overweight (BMI ≥ 25) tended to be higher in children with asthma than in healthy children (21.2% versus 5%; p=0.09). There tended to be more boys in the asthma group, consistent with the increased prevalence of asthma among boys. The FEV1 % predicted and FEV1/FVC ratio in children with asthma were less than healthy children (91.9% versus 98.9%; p=0.05; 81.6% versus 88.9%; p=0.002, respectively). 59.7% children with asthma had AHR to 4.5% saline solution. About 64% of children with asthma took ICS and the daily dose of ICS (IQR) was 250(0-800) μg.

Table 3.1.2: Asthma control score in children with asthma

<table>
<thead>
<tr>
<th>Characteristics (median, IQR)</th>
<th>Asthma control score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woken by asthma</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Symptom upon waking</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Activities limited by asthma</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>SOB due to asthma</td>
<td>0 (0-1)</td>
</tr>
<tr>
<td>Wheeze</td>
<td>0 (0-1)</td>
</tr>
<tr>
<td>SABA puffs per day</td>
<td>0 (0-1)</td>
</tr>
<tr>
<td>FEV1 % predicted</td>
<td>1 (0-1)</td>
</tr>
<tr>
<td>Overall score</td>
<td>0.5 (0.14-1)</td>
</tr>
</tbody>
</table>
Asthma control score was assessed in 64 children with asthma (97%). The children with asthma had well-controlled asthma, with a median (IQR) asthma control score of 0.5 (0.14-1). The median of all individual items was 0, except FEV₁% predicted. The median FEV₁% predicted was 1.

_Atopy_

A total of 85 subjects underwent skin prick tests with 4 allergens, which have been described above. Only one child with asthma refused to perform this test.

_Table 3.1.3: Characteristics of atopy in healthy children and children with asthma_

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Asthma</th>
<th>P value #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopy</td>
<td>50%</td>
<td>75.4%</td>
<td>0.03</td>
</tr>
<tr>
<td>Alternaria tenuis</td>
<td>30%</td>
<td>30.8%</td>
<td>0.95</td>
</tr>
<tr>
<td>Dust mite (DP)</td>
<td>45%</td>
<td>72.3%</td>
<td>0.02</td>
</tr>
<tr>
<td>Cockroach</td>
<td>30%</td>
<td>27.7%</td>
<td>0.8</td>
</tr>
<tr>
<td>Grass mix</td>
<td>30%</td>
<td>50.8%</td>
<td>0.1</td>
</tr>
</tbody>
</table>

# = Pearson Chi² Test  
DP: *Dermatophagoides pteronyssinus*

The prevalence of atopy in children with asthma was significantly higher than healthy children (75.4% versus 50%, p=0.03). Children with asthma had a significantly higher frequency of sensitization to Dust mite than healthy controls (72.3% versus 45%, p=0.02). The prevalence of Grass mix sensitization in children with asthma tended to be higher than in healthy children (50.8% versus 30%; p=0.1). However, no differences
were found in the prevalence of sensitization to *Alternaria* and Cockroach between healthy children and children with asthma.

*Airway hyperresponsiveness*

Airway hyperresponsiveness (AHR) is an important characteristic of asthma. None of the children in the healthy group demonstrated AHR to 4.5% saline solution while 59.7% of children with asthma had AHR in the current study.

*Fractional exhaled nitric oxide*

FeNO measurements were performed in both healthy children and children with asthma. FeNO results were recorded in 19 healthy controls and 51 children with asthma. Other data was lost due to inability to co-operate in FeNO measurements (n= 4) or technical problems (n=12).

*Figure 3.1.1: Median FeNO levels (ppb) in healthy children and children with asthma*
The median FeNO level (IQR) in the children with asthma was twofold higher than the FeNO level of the healthy children [24.8 (11.7-46.3) ppb versus 12.3 (7.9-21) ppb, \( p = 0.0025 \); Figure3.1.1].

FeNO and subject characteristics

*Table 3.1.4: Influence of subject characteristics on FeNO. r: correlation coefficient.*

*P-value for correlation between FeNO and subject variables*

<table>
<thead>
<tr>
<th>Subject Characteristic</th>
<th>N=19</th>
<th>N=51</th>
<th>N=70</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeNO level of healthy children</td>
<td>FeNO level of children with asthma</td>
<td>FeNO level of total children</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.34</td>
<td>0.32</td>
<td>0.35</td>
</tr>
<tr>
<td>Height</td>
<td>0.33</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>Weight</td>
<td>0.37</td>
<td>0.27</td>
<td>0.33</td>
</tr>
<tr>
<td>BMI</td>
<td>0.46</td>
<td>0.11</td>
<td>0.25</td>
</tr>
<tr>
<td>Gender</td>
<td>0.37</td>
<td>0.4</td>
<td>0.32</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>0.32</td>
<td>-0.24</td>
<td>-0.077</td>
</tr>
<tr>
<td>% FEV₁/FVC</td>
<td>0.37</td>
<td>-0.32</td>
<td>-0.16</td>
</tr>
</tbody>
</table>
Figure 3.1.2: Correlation between FeNO levels and age in children with asthma

\[(r = 0.32, p=0.02).\]

Figure 3.1.3: Correlation between FeNO levels and height of children with asthma

\[(r = 0.39, p=0.005).\]
Figure 3.1.4: Correlation between FeNO levels and weight of children with asthma

\[(r = 0.27, p = 0.055).\]

Figure 3.1.5: Correlation between FeNO levels and FEV₁ % predicted in children with asthma \[(r = -0.24, p = 0.09).\]
Figure 3.1.6: Correlation between FeNO levels and FEV₁/FVC ratio in children with asthma ($r = -0.32$, $p=0.02$).

Table 3.1.4 shows that FeNO levels in children were significantly increased with increased age ($r=0.35$, $p=0.0026$), height ($r=0.39$, $p=0.0009$), weight ($r=0.33$, $p=0.0052$) and BMI ($r=0.25$, $p=0.035$). For age, height and weight, the correlations with FeNO levels were of similar magnitude between healthy children and children with asthma, but only reached to statistical significance in asthma.

In healthy children, the FeNO levels were not significantly correlated with age ($r=0.34$, $p=0.15$), height ($r=0.33$, $p=0.16$), weight ($r=0.37$, $p=0.11$), gender ($p=0.37$) and lung function [FEV₁% predicted ($r=0.32$, $p=0.17$), FEV₁/FVC ratio ($r=0.37$, $p=0.11$)]. In children with asthma, FeNO levels were significantly correlated with age ($r=0.32$; $p=0.02$), height ($r=0.39$; $p=0.005$), weight ($r=0.27$; $p=0.055$), and FEV₁/FVC ratio ($p=0.02$). FeNO levels tended to be negatively related with FEV1 % predicted, with the
higher level of FeNO, the lower FEV₁% predicted (r=-0.24, p=0.09; Table 3.1.4; Figure 3.1.5). Similarly, FEV₁/FVC was negatively correlated with FeNO levels (r= -0.32, p=0.02; Table 3.1.4; Figure 3.1.6). The correlation of FeNO with BMI was significant in healthy children (r=0.46, p=0.048) but not in asthma.

The effects of demographic characteristics of childhood asthma including age, height, weight and sex on FeNO level of total children and children with asthma were assessed in multivariate analysis.

+ In total children:

\[ \text{FeNO (ppb)} = -114.3 - 2.6 \text{ sex} - 1.2 \text{ age} + 1.2 \text{ height} - 0.3 \text{ weight} \]

+ In children with asthma

\[ \text{FeNO (ppb)} = -302.9 + 8.6 \text{ sex} - 5.9 \text{ age} + 2.9 \text{ height} - 0.7 \text{ weight} \]

In multivariate analysis, this study found that FeNO levels were not related with age, height, and weight in the whole group, but significant increase in FeNO levels with increasing height in children with asthma (p=0.005) was reported.
FeNO level and atopy

*Figure 3.1.7: The relationship between median FeNO levels and atopy*

![Graph showing FeNO levels (ppb) for Healthy and Asthma groups, with bars indicating Non atopic and Atopic categories.]

The FeNO levels were significantly higher in atopic than in non-atopic children in both the control and asthma groups [18.05 (12.75-58.04) ppb versus 7.9 (7.3-10.6) ppb, p=0.002; 36.9 (20.3-53.3) ppb versus 10.6 (8.3-15.75) ppb, p=0.0001; respectively; Figure 3.1.7]. The FeNO level was highest in atopic childhood asthma. Conversely, no difference in FeNO levels was found between non-atopic healthy controls and non-atopic asthmatic children (7.9 ppb versus 10.6 ppb; p=0.16).
Table 3.1.5: Association between FeNO levels and atopic sensitization

<table>
<thead>
<tr>
<th>FeNO levels (median, IQR)</th>
<th>Healthy</th>
<th>Asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Atopy</td>
<td>18.05 (12.75-58.04)</td>
<td>7.9 (7.3-10.6)</td>
</tr>
<tr>
<td>Dust mite (Dp)</td>
<td>18.05 (12.75-58.04)</td>
<td>7.9 (7.3-10.6)</td>
</tr>
<tr>
<td>Alternaria tenuis</td>
<td>37.5 (14.15-60.9)</td>
<td>10.6 (7.6-12.6)</td>
</tr>
<tr>
<td>Cockroach mix</td>
<td>56.07 (15.1-60)</td>
<td>10.6 (7.6-12.6)</td>
</tr>
<tr>
<td>Grass-mix</td>
<td>35.59 (14.55-58.94)</td>
<td>10.6 (7.6-12.6)</td>
</tr>
</tbody>
</table>

Figure 3.1.8: The relationship between median FeNO levels and dust mite sensitization
Investigation of the relationship between FeNO levels and subjects who were sensitized to dust mite showed a similar result. Healthy children who had a positive reaction to dust mite had higher FeNO levels than those who had a negative reaction to dust mite [18.05 (12.75-58.04) ppb versus 7.9 (7.3-10.6) ppb, p=0.002]. Children with asthma who were sensitized to dust mite had significantly increased FeNO levels compared with those without dust mite sensitization [38.8 (22.6-53.3) ppb versus 11.4 (8.6-16.5) ppb, p=0.0001].

Healthy children who were sensitized to *Alternaria tenuis* had higher FeNO levels compared to those were not sensitized to *Alternaria tenuis* [37.5 (14.15-60.9) ppb versus 10.6 (7.6-12.6) ppb, p=0.01] whereas no difference in FeNO levels in children with asthma with and without *Alternaria tenuis* sensitization was observed [28 (21-39.17) ppb versus 19.8 (11.4-53.1) ppb, p=0.68].

FeNO levels were significantly higher in children who were sensitized to Cockroach and Grass mix compared with those without Cockroach and Grass sensitization in both healthy and asthmatic groups [56.07 (15.1-60) ppb versus 10.6 (7.6-12.6) ppb, p=0.04; 40 (22.6-53.3) ppb versus 18.1 (10.55-42.3) ppb, p= 0.017; 35.59 (14.55-58.94) ppb versus 10.6 (7.6-12.6) ppb, p=0.019; 34.95 (20.3-72.8) ppb versus 16.5 ( 9.8-41.2)ppb, p=0.016; respectively].
This study also investigated the relationships between the cumulative sizes of allergic reactions and FeNO levels. The cumulative size was calculated based on the average of the length of long axis and the length of its perpendicular of allergic weal size. There were no associations between FeNO levels and the weal size of *Alternaria* \((r=0.13, \ p=0.53)\), Cockroach mix \((r=0.009, \ p=0.97)\), and Grass mix \((r=0.17, \ p=0.32)\) reactions. However, there was a significant correlation between FeNO levels and the size of Dust mite reactions \((r=0.38, \ p=0.0054)\).
The median FeNO level (IQR) was 9.75 (7.6-13) ppb for non-atopic children, 17.3 (12.3-45.3) ppb for children who were sensitized to one allergen, 42.85 (24.8-78.6) ppb for children with two positive allergens and 37.6 (21-58.04) ppb for children with equal or more than three positive allergens. There was a significant correlation between FeNO levels and the total number of positive skin prick tests (p=0.0001). The FeNO levels were significantly higher in children who have $\geq 2$ positive allergen reactions.
FeNO and Airway hyperresponsiveness

*Figure 3.1.11: Comparison in FeNO levels in children with asthma with and without AHR*

FeNO levels were significantly related with AHR. The median FeNO level was 11.35 ppb (8-65-18.4) in children with asthma without AHR compared to 42.85 ppb (22.6-72.8) in those with AHR (p=0.0001). There was also a significantly negative correlation between FeNO levels and provocation dose of hypertonic saline solution (PD$_{15}$) ($r = -0.47; p =0.009$) (Figure 3.1.11).
The relationships between FeNO levels and FEV₁, FEV₁/FVC and AHR in whole group and children with asthma were assessed in multivariate analysis.

+ In total children

FeNO (ppb) = 7 + 1.0 FEV₁ – 0.4 FEV₁/FVC – 2.3 PD₁₅

+ In children with asthma

FeNO (ppb) = 5.9 + 1.1 FEV₁ – 0.5 FEV₁/FVC – 3.7 PD₁₅

In multivariate analysis, this study found a significantly negative correlation between FeNO levels and the provocation dose of hypertonic saline in the whole group (p=0.03) and children with asthma (p=0.012).
Exhaled breath condensate

Exhaled breath condensate collection was performed in both healthy children and children with asthma. EBC samples were collected in twenty healthy children (100%) and sixty five (98.5%) children with asthma. The median volume of EBC samples was 1050 (830-1290) μL. No difference in pH of exhaled breath condensate (EBC) was found between healthy children and children with asthma [7.35 (7.1-7.8) versus 7.4 (6.9-7.8); p=0.7].

EBC pH and subject characteristics

*Table 3.1.6: Influence of subject characteristics on EBC pH. r: correlation coefficient.*

<table>
<thead>
<tr>
<th>P-value for correlation between EBC pH and subject variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBC pH of healthy children</td>
</tr>
<tr>
<td>N=20</td>
</tr>
<tr>
<td>r</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Height</td>
</tr>
<tr>
<td>Weight</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>FEV₁ % predicted</td>
</tr>
<tr>
<td>% FEV₁/FVC</td>
</tr>
</tbody>
</table>
Table 3.1.6 shows that no correlations were observed between EBC pH and age, height, weight, BMI, gender, FEV$_1$% predicted, and FEV$_1$/FVC ratio in both healthy children and children with asthma.

**EBC pH and atopy**

EBC pH was similar between non-atopic and atopic healthy children [7.3 (7.0-7.9) versus 7.5 (7.1-7.7), p=0.65]. Also, no difference was found between non-atopic and atopic asthmatic children [7.5 (7.15-7.7) versus 7.4 (6.9-7.8), p=0.63].

**EBC pH and airway hyperresponsiveness**

Children with asthma with or without AHR showed a similar EBC pH results [7.4 (6.9-7.8) versus 7.45 (7.1-7.8), p=0.86].
### Induced sputum

#### Table 3.1.7: Induced sputum cell counts in the healthy children and children with asthma

<table>
<thead>
<tr>
<th>Variables (median, IQR)</th>
<th>Healthy (n=14)</th>
<th>Asthma (n=42)</th>
<th>P values +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell count x10^6/ml median (IQR)</td>
<td>2.16 (1.53-3.69)</td>
<td>2.43 (1.22-6.48)</td>
<td>0.19</td>
</tr>
<tr>
<td>Viability (%) median (IQR)</td>
<td>68 (57-75)</td>
<td>68 (50-85)</td>
<td>0.98</td>
</tr>
<tr>
<td>Quality, median (IQR)</td>
<td>20 (17-22)</td>
<td>17 (15-19)</td>
<td>0.015</td>
</tr>
<tr>
<td><strong>Cellular differential (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils, median (IQR)</td>
<td>12 (5-19.5)</td>
<td>13.38 (5.75-37)</td>
<td>0.36</td>
</tr>
<tr>
<td>Eosinophils, median (IQR)</td>
<td>0.25 (0-1)</td>
<td>3.25 (0.5-10.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Macrophages, median (IQR)</td>
<td>84.5 (75.25-90.75)</td>
<td>67.5 (39-83.25)</td>
<td>0.016</td>
</tr>
<tr>
<td>Lymphocytes, median (IQR)</td>
<td>1.25 (0.5-2)</td>
<td>0.75 (0.25-1.75)</td>
<td>0.18</td>
</tr>
<tr>
<td>Epithelial cells, median (IQR)</td>
<td>1.5 (0.5-2.75)</td>
<td>2 (0.25-4.25)</td>
<td>0.58</td>
</tr>
</tbody>
</table>

IQR= Inter-quartile range  
+ = Mann-Whitney Test

Adequate samples were obtained from 63.6% of children with asthma and 65% of healthy children after sputum induction. There were no differences in the sputum total cell counts, neutrophils, lymphocytes, and epithelial cells between healthy children and children with asthma. Sputum eosinophils in children with asthma were significantly higher than in healthy children (3.25% versus 0.25%; p=0.01) whereas sputum macrophages in the healthy children were higher than in the children with asthma (84.5% versus 67.5%; p=0.016). Examples of sputum cellular differentials are shown in figure 3.1.13 and 3.1.14.
Figure 3.1.13: Induced sputum in healthy children: cytospin stained with May Grunwald Giemsa, demonstrates normal proportions of macrophages, lymphocytes and neutrophils.
Figure 3.1.14: Induced sputum in asthma: cytospin demonstrating eosinophils, macrophages and neutrophils.
Sputum eosinophils and subject variables

Table 3.1.8: Influence of subject characteristics on % sputum eosinophils. \( r \): correlation coefficient. \( P \)-value for correlation between % sputum eosinophils and subject variables

<table>
<thead>
<tr>
<th></th>
<th>Sputum eosinophils of healthy children N=13</th>
<th>Sputum eosinophils of children with asthma N=42</th>
<th>Sputum eosinophils of whole group N=55</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )  ( P ) value</td>
<td>( r )  ( P ) value</td>
<td>( r )  ( P ) value</td>
</tr>
<tr>
<td>Age</td>
<td>0.02  0.95</td>
<td>0.079  0.62</td>
<td>0.069  0.62</td>
</tr>
<tr>
<td>Height</td>
<td>0.0056  0.99</td>
<td>-0.0051  0.97</td>
<td>-0.009  0.95</td>
</tr>
<tr>
<td>Weight</td>
<td>-0.025  0.94</td>
<td>0.088  0.58</td>
<td>0.094  0.5</td>
</tr>
<tr>
<td>BMI</td>
<td>0.12  0.69</td>
<td>0.14  0.36</td>
<td>0.18  0.18</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.17  0.88</td>
</tr>
<tr>
<td>FEV(_1)% predicted</td>
<td>0.32  0.29</td>
<td>-0.14  0.36</td>
<td>-0.11  0.43</td>
</tr>
<tr>
<td>% FEV(_1)/FVC</td>
<td>0.55  0.061</td>
<td>-0.42  0.0052</td>
<td>-0.32  0.017</td>
</tr>
</tbody>
</table>
Figure 3.1.15: Relationship between % sputum eosinophils and % FEV₁/FVC ratio in children with asthma (r=-0.42, p=0.0052)

In healthy children, no relationships between % sputum eosinophils and subject characteristics such as age (r=0.02, p=0.95), height (r=0.0056, p=0.99), weight (r=-0.025, p=0.94), BMI (r=0.12, p=0.69), sex (p=0.17), FEV₁ % predicted (r=0.32, p=0.29) and FEV₁/FVC ratio (r= 0.55, p=0.061) were reported. In children with asthma, % sputum eosinophils were also not correlated with subject characteristics such as age (r=0.079, p=0.62), height (r=-0.0051, p=0.97), weight (r= 0.088, p=0.58), BMI (r=0.14, p=0.36), sex (p=0.88), and FEV₁ % predicted (r=-0.14, p=0.36). However, % sputum eosinophils were negatively correlated with % FEV₁/FVC ratio in children with asthma (r=-0.42, p=0.0052; Table 3.1.8; Figure 3.1.15).
Sputum eosinophils and atopy

*Figure 3.1.16: Relationship between sputum eosinophils and atopy in healthy children and children with asthma*

Children with atopy had significantly higher % sputum eosinophils compared to non-atopic children [2.925% (0.75-10.5) versus 0.125% (0-0.5); p=0.008]. In the healthy children, a small but significant increase in % sputum eosinophils in atopic subjects compared with non-atopic subjects was observed [0.75% (0.625-1.5) versus 0.25% (0-0.5), p=0.03]. Similarly, children with atopic asthma had higher % sputum eosinophils than childhood asthma without atopy [4.25% (1-15.25) versus 0.5% (0-0.75), p=0.01].
In children with asthma, the median % sputum eosinophils (IQR) for subjects with AHR was 3.5% (0.75-13.75) compared with 0.5% (0-2.73) for subjects without AHR. The difference in sputum eosinophils was significant between two groups (p=0.004).

**Figure 3.1.18: Correlation between % sputum eosinophils and PD15 in children with asthma** (r=-0.42, p=0.034).
Sputum eosinophils were also negatively associated with PD15 with hypertonic saline (r= -0.42, p=0.034).

Sputum neutrophils and subject variables

Table 3.1.9: Influence of subject characteristics on sputum neutrophils. r: correlation coefficient. P-value for correlation between % sputum neutrophils and subject variables

<table>
<thead>
<tr>
<th>Subject Characteristic</th>
<th>Sputum neutrophils of healthy children N=13</th>
<th>Sputum neutrophils of children with asthma N=42</th>
<th>Sputum neutrophils of whole group N=55</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P value</td>
<td>r</td>
</tr>
<tr>
<td>Age</td>
<td>-0.3</td>
<td>0.32</td>
<td>0.26</td>
</tr>
<tr>
<td>Height</td>
<td>-0.49</td>
<td>0.087</td>
<td>0.24</td>
</tr>
<tr>
<td>Weight</td>
<td>-0.48</td>
<td>0.095</td>
<td>0.24</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.36</td>
<td>0.22</td>
<td>0.14</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>FEV1% predicted</td>
<td>-0.15</td>
<td>0.62</td>
<td>-0.02</td>
</tr>
<tr>
<td>% FEV1/FVC</td>
<td>-0.17</td>
<td>0.57</td>
<td>0.075</td>
</tr>
</tbody>
</table>

In healthy children, no significant relationships were found between % sputum neutrophils and subject characteristics such as age (r=-0.3, p=0.32), height (r=-0.49, p=0.087), weight (r= -0.48, p=0.095), BMI (r= -0.36, p=0.22), gender ( p=0.18), FEV1% predicted ( r= -0.15, p=0.62), and % FEV1/FVC ratio ( r=-0.17, p=0.57). Also, in children
with asthma, % sputum neutrophils were not correlated with subject characteristics such as age (r=0.26, p=0.092), height (r=0.24, p=0.13), weight (r=0.24, p=0.13), BMI (r=0.14, p=0.38), gender (p=0.21), FEV1% predicted (r= -0.02, p=0.9), and % FEV1/FVC ratio (r=0.075, p=0.63).

Sputum neutrophils and atopy
There were no differences in % sputum neutrophils between subjects with and without atopy in both healthy children and children with asthma [5.88% (4-88- 13.88) versus 19.5% (12.5- 20.75), p=0.12; 15.75% (5.75-37) versus 8% (2.75-58), p= 0.46; respectively].

Sputum neutrophils and AHR
There was no difference in % sputum neutrophils in children with asthma with and without AHR [17 % (6.3-43.75) versus 6.75% (4.75- 27.46), p=0.28].
While sputum samples were obtained from approximately 64% of children, 81.4% of those were able to successfully perform FeNO measurement, and EBC samples were collected in almost all children (98.8%). There were significant differences in the success rate of the various methods (p<0.0001).
Relationship between FeNO and sputum results

Table 3.1.10: Correlation between FeNO levels and sputum results

<table>
<thead>
<tr>
<th>Sputum results</th>
<th>FeNO of healthy children N=13</th>
<th>FeNO of children with asthma N= 42</th>
<th>FeNO of whole children N=55</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Eosinophils</td>
<td>0.59 0.045</td>
<td>0.54 0.001</td>
<td>0.56 0.0001</td>
</tr>
<tr>
<td>Eosinophil absolute (x10^4/ml)</td>
<td>0.19 0.57</td>
<td>0.5 0.008</td>
<td>0.45 0.0042</td>
</tr>
<tr>
<td>% Neutrophils</td>
<td>-0.6 0.04</td>
<td>0.09 0.6</td>
<td>-0.02 0.89</td>
</tr>
<tr>
<td>Neutrophil absolute (x10^4/ml)</td>
<td>-0.6 0.05</td>
<td>-0.13 0.5</td>
<td>-0.22 0.19</td>
</tr>
</tbody>
</table>
Figure 3.1.20: The correlation between FeNO levels and % sputum eosinophils in healthy children ($r = 0.59$, $p = 0.045$)

Figure 3.1.21: The correlation between FeNO levels and % sputum neutrophils in healthy children ($r = -0.6$, $p = 0.04$)
FeNO levels were significantly related with % sputum eosinophils in healthy children ($r=0.59$, $p=0.045$) (Table 3.1.10, figure3.1.20). Also both % sputum neutrophils and absolute sputum neutrophils were negatively correlated with FeNO levels ($r=-0.6$, $p=0.04$; $r=-0.6$, $p=0.05$; respectively) (Table 3.1.10, figure3.1.21, figure3.1.22). However, the number of healthy children with adequate sputum samples for analysis was small (n=12).
Airway inflammation with eosinophils is accepted as a fundamental characteristic of asthma. The levels of FeNO were significantly correlated with the percentage of sputum eosinophils in children with asthma. The correlation between FeNO levels and % sputum eosinophils in children with asthma is shown in Figure 3.1.23 (r = 0.54, p = 0.001). The correlation between FeNO levels and absolute sputum eosinophils in children with asthma is shown in Figure 3.1.24 (r = 0.5; p = 0.008).
eosinophils ($r=0.54$, $p=0.001$) (Table 3.1.8; figure3.1.23), and the absolute sputum eosinophil counts ($r=0.5$; $p=0.008$) (Table 3.1.8, figure3.1.24) in children with asthma. Neither % sputum neutrophils nor absolute sputum neutrophils were correlated with FeNO levels in children with asthma.

Investigation of the whole group found that FeNO levels were significantly correlated with % sputum eosinophils ($r=0.56$, $p=0.0001$) and absolute sputum eosinophil counts ($r=0.45$, $p=0.0042$), but neither % sputum neutrophils ($r=-0.02$, $p=0.89$) nor absolute sputum neutrophil counts ($r=-0.22$, $p=0.19$).

Relationship between EBC pH and sputum results

*Figure 3.1. 25: Correlation between % sputum eosinophils and EBC pH in children with asthma ($r=0.09$, $p=0.56$)*

There was no correlation between % sputum eosinophils and EBC pH in children with asthma ($r=0.09$, $p=0.56$; Figure 3.1.25).
Similarly, % sputum neutrophils were not correlated with EBC pH in children with asthma \( (r=0.14, p=0.37) \); Figure 3.1.26).

Relationship between FeNO and EBC pH

Figure 3.1. 27: Correlation between FeNO levels and EBC pH in healthy children \( (r=0.3, p=0.2) \)
There were no correlations between FeNO levels and EBC pH in both healthy children and children with asthma ($r=0.3, p=0.2$; $r=-0.086, p=0.55$; respectively).

**Sputum cytokines**

**Table 3.1.11: Comparison in sputum cytokines between healthy children and children with asthma**

<table>
<thead>
<tr>
<th></th>
<th>Healthy (13)</th>
<th>Asthma (47)</th>
<th>P value $^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 (median, IQR; ng/ml)</td>
<td>0.98 (0.7-1.8)</td>
<td>1.95 (0.89-5.35)</td>
<td>0.21</td>
</tr>
<tr>
<td>TNF-α (median, IQR; pg/ml)</td>
<td>0</td>
<td>250 (202-2392)</td>
<td></td>
</tr>
</tbody>
</table>

$^+$ = Mann-Whitney Test
The median IL-8 (IQR) in healthy children was 0.98 ng/ml (0.7-1.8) whereas it was 1.95 ng/ml (0.89-5.35) in children with asthma. IL-8 in children with asthma was not significantly different compared to healthy children (p=0.21).

The limit of detection of TNF-α values in sputum samples was 62.4 pg/ml. In all sputum samples which were below the detection threshold, the lower limit of detection was assigned, and this applied to most samples as most of them had TNF-α less than 62.4 pg/ml.

There were 10 healthy children and 29 children with asthma who had sputum TNF-α measured, but only 4 children with asthma had sputum TNF-α more than 62.4 pg/ml. This means sputum TNF-α was detected in only 13.7% of children with asthma. No sputum TNF-α result was detected in healthy children.

Table 3.1.12: Correlation between IL-8 with lung function and sputum cells

<table>
<thead>
<tr>
<th></th>
<th>Healthy children</th>
<th></th>
<th>Children with asthma</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation with IL-8</td>
<td>P value</td>
<td>Correlation with IL-8</td>
<td>P value</td>
</tr>
<tr>
<td>FEV₁% predicted</td>
<td>-0.31</td>
<td>0.3</td>
<td>-0.13</td>
<td>0.39</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>-0.34</td>
<td>0.25</td>
<td>0.06</td>
<td>0.69</td>
</tr>
<tr>
<td>% sputum neutrophils</td>
<td>0.27</td>
<td>0.42</td>
<td>0.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>% sputum eosinophils</td>
<td>-0.52</td>
<td>0.1</td>
<td>-0.04</td>
<td>0.78</td>
</tr>
</tbody>
</table>
Table 3.1.12 shows the correlation between IL-8 with lung function and sputum cells in healthy children and children with asthma. There were no correlations between IL-8 and FEV$_1$ % predicted, FEV1/FVC ratio, % sputum neutrophils and % sputum eosinophils in healthy children. Similarly, no correlations were indicated except % sputum neutrophils in children with asthma. This study found a significant correlation between IL-8 and % sputum neutrophils ($r=0.7$, $p=0.0001$).
Discussion

The study was conducted in both healthy children and children with asthma with matched age, height, and weight distribution. The results confirm the differences in lung function as well as airway markers between healthy and asthmatic children, and provide a valuable insight into how the various inflammatory markers perform in describing airway inflammation in children.

1- Lung function

Asthma in children is often diagnosed based on clinical features rather than lung function, especially in young children (442). However, lung function testing can be performed and be useful to diagnose and manage asthma in school-aged children and adolescents (156, 442). The mean FEV$_1$/FVC ratio and FEF$_{25-75\%}$ are significantly less in childhood asthma compared with control subjects (443). According to GINA 2005, FEV$_1$ in children over 5 years with infrequent episodic and frequent episodic asthma is at least 80% predicted (444). Fortunately, infrequent intermittent and frequent intermittent asthma are the predominant patterns, which occur in approximately 95% of children with asthma (156). In this study, although FEV$_1$ % predicted and % FEV$_1$/FVC ratio in children with asthma were lower compared with healthy children, they were still within the normal range. The distribution of clinical pattern of children with asthma can explain why lung function in childhood asthma is often normal during stable periods.

2- Atopy

Atopy is more common in childhood asthma. Atopy has been seen as the single strongest risk factor for the development of asthma (445). Allergy has an important role in
initiating and maintaining the inflammatory process in asthma (446). A total of 80% of children with asthma have positive skin-prick tests to environmental allergens (43).

Subjects with asthma are often sensitized to aeroallergens. Indoor aeroallergens such as dust mite, cockroaches, and mould spores and outdoor aeroallergens such as pollen, grass and mould spores are typically associated with asthma. The principal dust mite in Australia and New Zealand is *Dermatophagoides pteronyssinus* (*Dp*) (54). High house dust mite allergen levels are found in humid, subtropical regions whereas high *Alternaria* allergens have been found in a dry, rural region (49). A study in 805 children in Lismore (a coastal region) and 770 children in Moree/Narrabri (inland region) of northern New South Wales found that the prevalence of Dust mite sensitization was similar between the two areas, but the prevalence of children who were sensitized to *Alternaria tenuis* was significantly higher in children living in the inland region compared to children living in the coastal region (49). Cockroach allergen levels are higher in older inner city houses and in tropical areas of Australia (54). Grass pollen is recognized as a second major allergen after house dust mite in the development of asthma (50).

This study confirms that the prevalence of atopy in children with asthma was significantly higher than healthy children. Newcastle is a sub-tropical and coastal city, and this explains why the prevalence of dust mite sensitivity was high in both healthy children and children with asthma. It also explains the low percentages of children with asthma who were sensitized to *Alternaria tenuis*. In children with asthma, the prevalence
of dust mite sensitization was higher than in healthy children, suggesting this is the dominant allergic trigger in this population.

3- Fractional exhaled Nitric oxide

FeNO testing was performed in both healthy children and children with asthma. The measurement of FeNO using the single breath online-method is feasible (447) and may reflect airway inflammation (368). FeNO measurement had also the potential to assess asthma control in children (448). This study confirms previous reports that FeNO levels were significantly higher in childhood asthma compared to healthy children (368, 443). A study in 155 Australian children aged 6-18 years found that children with current asthma had higher FeNO levels compared to non asthmatic children (24.5ppb versus 11ppb, p<0.001), also those with reported recent wheeze had increased FeNO levels compared to those without recent wheeze (16.6 ppb versus 10.8 ppb, p=0.01)(449).

3.1-Subject characteristics

Previous studies have reported an inconsistent correlation between FeNO levels and subject characteristics. Malmberg et al performed FeNO testing in 114 non-atopic and non smoking healthy children, which demonstrated significant relationships with age, standing height, weight, and body surface area, but not with gender (450). A study in 405 healthy children aged 4-17 years found FeNO levels were significantly increased with age (451). Latzin et al also reported a correlation between FeNO and age, but not with gender in healthy children (452). Other studies have confirmed an increase in FeNO levels with age (453, 454). In children with asthma, Avital et al showed that FeNO was
age dependent, with lower FeNO levels at small ages (455). In contrast, Nelson et al found that FeNO levels did not vary by age, sex, height or weight in both healthy children and children with asthma (443). Also no significant correlation was demonstrated between FeNO and age and height in 159 healthy children in Baraldi’s study (456). Similarly, there was no difference in FeNO levels between boys and girls in both healthy children and children with asthma (443).

In a univariate analysis, the current study did not find any correlations between FeNO levels with age, height, and weight in healthy children. But FeNO levels were weakly correlated with age, height and weight in children with asthma. The correlations may have been shown in children with asthma because of the wide range of FeNO levels compared with healthy children. Investigation of the whole group (both healthy and asthma) indicated a small increase in FeNO levels with increasing age, height, weight suggesting FeNO levels in children maybe partly dependent on children’s characteristics. In multivariate analysis, this study found that FeNO levels were not related with age, height, and weight in the whole group, but significant increase in FeNO levels with increasing height in children with asthma. The different findings may be explained by the small number of healthy children (n=19) which may not provide enough power to detect an effect. The findings also confirmed that height was the important independent predictors of increased FeNO levels.

The current literature reports variable effects of age and height on FeNO levels. When FeNO levels were dependent on age, the increasing age resulted in their elevated FeNO
levels. Taylor *et al* suggested that increasing age was related to increasing airway surface area, or a longer constant exhalation flow rate, or an increase in induction of NOS secondary to recurrent stimulation, which may have caused the increased FeNO levels with increasing age (457). Children were still growing and an increase in age was correlated with increased height. Several recent studies have attempted to establish the normal ranges of FeNO levels in healthy children (451). The estimation of FeNO levels in children adjusted for age and height may provide a good indicator to distinguish between subjects with and without normal FeNO levels.

3.2-Lung function

No correlation between FeNO levels and FEV₁ was found in healthy children (443, 452). Baraldi *et al* also reported with no association between spirometric data and FeNO levels in healthy subjects of 6-15 years (456). In children with asthma, studies have found a negative correlation between FEV₁ % predicted and FeNO levels (458-460). This study found a trend to a negative correlation between FEV₁ % predicted and FeNO levels, but this failed to reach statistical significance (p=0.09). This may be because the FEV₁ % predicted of children with asthma was still within normal range while FeNO levels were increased. The FeNO levels were also reported to be negatively related with FEV₁/FVC ratio in asthmatic subjects (461). A similar result was found in this study, which supports the concept that increased inflammation occurs in children with more severe airflow obstruction.
3.3- Atopy

Non-atopic subjects have lower FeNO levels compared to atopic subjects, in both healthy children and children with asthma (368, 449). FeNO levels may distinctly differ in atopic and non-atopic subjects (368). Both healthy children and children with asthma who were sensitized to house dust mite had significantly higher FeNO compared with those who were not sensitized to house mite (368, 462).

However, other studies found that atopic and non-atopic children without asthma had similar FeNO levels (463). Latzin et al conducted a study in 107 healthy children and found no correlation between FeNO levels and atopic individuals (452). This study determined allergic sensitization with 16 allergens including egg, milk, nut mixture, grass mix, birch, alder, willow, rye, hazel, herbs mixture, epithelia from dog, cat, horse, rabbit and goose, *Alternaria alternata, Aspergillus niger, Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. We suggest that the difference in types of allergen sensitization may explain the difference in increased FeNO levels. Previous publications indicated that FeNO levels were often increased in subjects who were sensitized to some specific aeroallergen such as dust mite rather than in subjects who were sensitized to other allergens (368). Leuppi *et al* conducted a study in 235 children aged 8-14 years found that increased FeNO levels were associated with sensitization to house dust mite but not with grass pollen or cat dander (464). Subjects who were sensitized to grass-pollen only had lower FeNO levels compared to subjects with mono-sensitized to dust-mite (368). Olin *et al* suggested that atopic subjects who were not being exposed to a relevant allergen showed normal FeNO levels (463). In Newcastle, where our subjects
lived, dust mite is a perennial allergen and children who were sensitized would have ongoing exposure. It seems likely that both sensitization and exposure are required to lead to increased FeNO.

Interestingly, some studies have reported a correlation between the FeNO level and the weal size of allergic reaction. The FeNO levels were correlated with the weal size of atopy for both asthmatic and nonasthmatic subjects (465). Barreto et al found a weak but significant correlation between FeNO levels with the weal size for Dust mite, cat allergen, mix grass pollen reactions, as well as the sum of all positive weals (368). In subjects who were sensitized to Dust mite, the weal size for dust mite was correlated with exhaled NO levels (466). The current study demonstrated a correlation between the weal sizes of Dust mite reactions and FeNO levels, but not with other allergens. The findings suggest that the population in Newcastle who were sensitized and exposed to Dust mite were experiencing active airway inflammation, as reflected by elevated FeNO. Subjects with greater degrees of allergic sensitization, as measured by increased weal sizes, would also have increased airway responses to allergen, including increased airway inflammation.

This study also found that subjects who were sensitized to dust mite, cockroach and grass mix had significantly higher FeNO levels compared with subjects who were not atopic in both healthy children and children with asthma. The findings may be explained by the high prevalence of dust mite sensitization in this population in both healthy and asthmatic groups. Moreover, atopic subjects were often sensitized to multiple allergens. The
combination in multiple allergic sensitizations in atopic subjects leads to difficulty to evaluate the effects of each allergen on FeNO levels.

Previous studies have also demonstrated that the increased FeNO levels were correlated with the number of positive skin reactions (461, 467). Franklin et al performed FeNO measurements in 157 healthy children aged 7-13 years. There were significantly different FeNO levels in children with different positive allergen reactions, as FeNO levels were 7.2 ppb in children with negative skin prick tests, 10.9 ppb in children with one positive reaction, and 20.1 ppb in children with two or more skin reactions (p<0.001) (453). Our study has found that there was a strong correlation between FeNO and the number of positive allergen skin prick tests. FeNO levels were significantly higher in children with ≥ two positive allergen reactions.

In sum, FeNO levels in children with asthma were higher than in healthy children, but not all children with asthma had high FeNO levels. The increased FeNO levels were dependent on the number and type of allergic reactions (368). The combination of sensitization and exposure to specific allergens can contribute to the raised FeNO levels.

Elevated FeNO levels in atopic subjects have been confirmed. However, the difference in FeNO levels between non-atopic asthma and atopic asthma raises a question of whether FeNO is a marker of atopy, rather than a marker of airway inflammation. It seems likely that there is an effect of both atopy and asthma leading to elevated FeNO levels. The
highest FeNO levels were seen in atopic asthmatic children, suggesting that the two variables interact to produce high FeNO levels.

4-Airway hyperresponsiveness

AHR is one of the clinical characteristics of asthma, and the measurement of AHR is widely used as a diagnostic test for asthma. Although increased airway responsiveness is usually associated with the diagnosis of asthma (430, 468), not all subjects with elevated airway responsiveness have asthma. On the other hand, not all children with asthma show AHR (7).

This study found that about 60% children with asthma had AHR. FeNO levels were significantly higher in childhood asthma with AHR compared to childhood asthma without AHR. There was also negative correlation between FeNO levels and provocation dose of hypertonic saline solution.

In children with asthma, the FeNO levels of those with AHR were significantly higher than of those without AHR (464, 469). In subjects with AHR, there was a significant correlation between FeNO levels and PC_{20} (362) and the dose response ratio (DRR) (464) to histamine. Jatakanon et al found a significant correlation between exhaled NO and PC_{20} to methacholine (470). However, other studies did not find any relationships between FeNO levels and AHR (467, 471, 472). Thomas et al found no correlation between FeNO levels and PD_{15} to hypertonic saline (467). In fact, this study reported the increased FeNO levels with elevated sensitivity to hypertonic saline, however only a
small number of children (n=14), accounting for 14.4% of study subjects, had achieved a saline PD15. In addition, this study was conducted in both Australian children and those who had migrated from more than 20 ethnic backgrounds. The effects of new environment on AHR as well as other inflammatory makers in asthma such as FeNO levels, sputum eosinophils are not clear.

Previous observations reported that the relationship between FeNO and AHR was only seen in atopic children (449, 473), suggesting the increased levels of FeNO and AHR occurrence were one of the phenotypes of asthma (with atopy, AHR, and raised FeNO levels) (474). In addition, the elevated FeNO levels combined with atopy and AHR were independent from asthma symptoms (449). The good relationship between atopy and AHR was well established (58, 475), but the mechanism of elevated FeNO in atopic asthmatic subjects with AHR is still unclear. Both genes and environment are suggested as necessary to establish the phenotypes of asthma (476). Variations in the NOS1 gene may contribute to atopy in children (477). In addition, both FeNO and airway responsiveness are significantly effected by genes (478). Franklin et al suggested that the increased FeNO in this phenotype may be due to genetic variations in Nitric oxide synthase (NOS) genes (449). In subjects with asthma, a correlation between a polymorphism in the NOS1 gene and variations in FeNO was reported (476, 479). Also the NOS1 isoform had an important role in the regulation of AHR in animal models (480), suggesting an effects of NOS genes in the relationship between increased FeNO and AHR in atopic subjects. FeNO levels are suggested as a marker of airway inflammation (467, 470, 481) and AHR is often associated with airway inflammation
increased FeNO and AHR occurrence in subjects with atopic asthma (473).

5- Exhaled breath condensate

Exhaled breath condensate collection is a non invasive method to assess airway inflammation in asthma (436, 482). The method is feasible and safe for children aged 4 years and above, with a success rate of up to 100% (418).

The pH of the airway in healthy controls is slightly alkaline (482). Subject characteristics such as age, gender, race did not affect EBC pH (483, 484). Acidification of the airway due to the inflammatory process in subjects with asthma may result in a reduced EBC pH. However, a study demonstrated that EBC pH values did not differ between 562 healthy children and 54 children with asthma (485). Some studies have found a lower value of EBC pH in stable asthma compared with controls (486, 487) whereas Ojoo et al reported a normal EBC pH level in stable asthma (436). There was a trend for lower EBC pH in subjects with current wheeze compared to subjects without wheeze (485). Hunt et al found that pH of EBC of subjects with acute asthma was significantly lower than control subjects but it was normalized by corticosteroid therapy (488). One study reported a lower EBC pH among patients with moderate to severe persistent asthma than intermittent asthma (435).

Endogenous airway acidification mostly depends on the amount of acid provided to the airway by inflammatory cells. Kostikas suggested that when the inflammatory process in
asthma was well controlled, EBC pH remained within normal range. If cellular inflammation was activated, the pH of airway started to decrease (486).

This study observed no difference in EBC pH between healthy children and children with asthma. These subjects were selected to be stable, with about 30% children with infrequent asthma and approximately 64% of childhood asthma were regularly taking ICS. These intermittent, stable asthma and ICS use may lead to normal EBC pH in childhood asthma. However, there was evidence of active eosinophilic inflammation observed with increased sputum eosinophils and FeNO. In addition, pH was significantly lower in BAL samples than EBC samples (6.4 versus 7.35, p <0.001) (489). The results suggest that EBC pH is insensitive marker for eosinophilic inflammation in children with stable asthma.

EBC pH was similar between atopic and non atopic subjects despite they were healthy or asthmatic children (490). Nicolaos et al have reported no consistent association between EBC pH and lung function, AHR, and FeNO levels (485). Similarly, this study did not show any correlation between EBC pH with % FEV$_1$ predicted, % FEV$_1$ /FVC ratio, AHR, atopy and FeNO levels.

6-Airway inflammation

Eosinophils are recognized as a key cell in the airway of subjects with asthma (165). Eosinophils are seldom present in sputum samples from normal subjects. Sputum eosinphils were significantly higher in both stable asthma and exacerbations of
childhood asthma compared to healthy children (172, 491, 492). The current study showed that the median sputum eosinophil count in children with stable asthma was 3.25% compared to 0.25% in healthy children.

6.1- Sputum eosinophils and AHR
Both sputum eosinophils and AHR are characteristics of subjects with asthma. Eosinophils were significantly correlated with airway responsiveness to hypertonic saline solution in adults (200). A significant correlation between % sputum eosinophils and $PC_{20}$ to methacholine was reported by Jatakanon (470). Our study confirms the previous reports, with significantly higher sputum eosinophils in subjects with AHR compared to subjects without AHR. Also, a significant correlation between sputum eosinophils and saline PD$_{15}$ was found, demonstrating that these mechanisms are present in childhood asthma.

6.2- Sputum eosinophils and FeNO
This study found that FeNO levels were significantly increased with an increase in sputum eosinophils in both healthy children and children with asthma. Previous studies reported positive correlations between FeNO levels and both sputum eosinophils (467, 470) and sputum ECP in children with asthma (467). Similarly, FeNO levels were also correlated with percentages of eosinophils and ECP in the BAL fluid in childhood asthma (481). The findings suggested that FeNO may be a marker of airway inflammation in asthma (467, 470, 472, 481).
In asthmatic subjects, the NO derived from airway epithelial cells may be a mechanism for amplifying airway inflammation through the inhibition of Th1 cells, resulting in an increase in the number of Th2 cells and cytokines including IL-4, which plays a critical role for IgE expression, and IL-5, which is important in recruitment of eosinophils into the airway (493).

On the other hand, no correlations were reported between FeNO levels and other inflammatory cells in children with asthma (481). This study also did not find any correlation between sputum neutrophils and FeNO levels in children with asthma.

In summary, raised FeNO identified individuals with atopy and increased airway responsiveness regardless of having a diagnosis of asthma or asthmatic symptoms (464, 474). However, FeNO levels were only increased in subjects who were sensitized to specific allergens such as house dust mite which were likely associated with AHR (58, 475), and symptoms of asthma (58, 494) which suggest that FeNO may reflect airway inflammatory disorders that are the main feature of asthma. Leuppi hypothesized that exposure to house dust mite in asthmatic subjects with dust mite sensitization were likely to cause airway inflammation, which was related to increased FeNO levels (464). A significant correlation between FeNO levels and airway eosinophilia also support that FeNO levels may reflect active allergic airway inflammation in asthma.

However, not all children with asthma had increased sputum eosinophils, also with the increased FeNO. The findings also raise a question whether or not there are two subtypes
of airway inflammation in children with asthma, one with lower sputum eosinophils, no AHR, and normal FeNO levels and another with higher sputum eosinophils, AHR and high FeNO levels. This question will be addressed in chapter II.

6.3- Sputum cells and EBC pH

There was no correlation between neither the percentage of sputum eosinophils nor the percentage of sputum neutrophils and EBC pH in both healthy subjects and subjects with asthma (495). This study also did not find any relationships between either sputum eosinophils or sputum neutrophils with EBC pH in both healthy children and children with asthma.

7- Sputum cytokines

Cytokines plays an important role in establish and maintain airway inflammation in subjects with asthma. Cytokines can promote the growth and differentiation of inflammatory cells (including eosinophils and mast cells). They also activate airway inflammatory process and locate in the airways (164).

On the other hand, inflammatory cells such as monocytes and macrophages can enhance airways inflammation in subjects with asthma by elevated cytokine production. Hallsworth et al found that after stimulation by LPS, macrophages from asthmatic subjects released threefold more TNF-α and fourfold more IL-8 compared with macrophages from control subjects (496). Azevedo et al showed that alveolar
macrophages from infants who suffered from severe wheezing were activated to release increased amounts of TNF-α (497).

Defining the cytokine profile in induced sputum can evaluate the inflammatory state of the airways. IL-8 is known as a cytokine, which is potential to be chemoattractant for neutrophils, which explains the significant correlation between sputum neutrophils and IL-8. In subjects with acute asthma, a prominent neutrophilic inflammation was often accompanied with an increase in IL-8 levels (162). During asthma exacerbations, epithelial damage was associated with activated neutrophils, increased IL-8 and neutrophil elastase, but not eosinophils (498). The increased neutrophil recruitments in the airway lumen and in the surrounding airway tissue were also observed (498). Not only in acute asthma, neutrophil inflammation with increased IL-8 were found in severe asthma (189, 499).

TNF-α is one of the inflammatory markers, which are responsible for neutrophil recruitment in the airways (500). In fact, low concentrations of TNF-α were detected in induced sputum of healthy subjects (501). In asthma, an increase in TNF-α levels was associated with neutrophil numbers (500).

Liu et al reported that the levels of serum IL-8 and TNF-α in the asthmatic patients during acute attack periods were higher than in the remission periods and healthy people. They also found the higher serum IL-8 and TNF-α levels in subjects with stable asthma compared to healthy controls, but they were not significant difference (502).
In stable asthma, sputum with elevated eosinophils, but not neutrophils were described (426, 491), that results in no significant difference in IL-8 and TNF-α levels compared to healthy subjects.

**Conclusion**

Children with asthma show differences in both clinical pattern (decreased lung function, increased atopic status, AHR) and pathological pattern (increased FeNO levels, and elevated sputum eosinophilia) compared with healthy children.

The non-invasive methods such as FeNO measurement, EBC collection, and sputum induction are safe and feasible methods to investigate airway disorders in both healthy children and children with asthma. FeNO measurement and induced sputum cell counts can be used to detect the eosinophilic inflammation that occurs in asthma.
CHAPTER II

AIRWAY INFLAMMATORY PHENOTYPES IN CHILDREN WITH ASTHMA

Introduction

The airway inflammatory response in asthma is complex and current guidelines describe asthma as a disorder where many cells and cellular elements play a role (439). Atopy and eosinophilic bronchitis are important in asthma, however, only about 50% of asthma can be attributed to eosinophilic airway inflammation (160). This suggests a role for non-eosinophilic mechanisms in asthma.

Heterogeneity of the inflammatory response is important in adult asthma, where non-eosinophilic forms of asthma are relatively common and less responsive to corticosteroids. Simpson et al have categorized airway inflammation into four subtypes based on sputum eosinophil and neutrophil proportions (161). The subtypes were eosinophilic asthma (EA) with increased sputum eosinophils, neutrophilic asthma (NA) with increased sputum neutrophils, mixed granulocytic asthma (MGA) in which both sputum eosinophils and neutrophils were increased, and paucigranulocytic asthma (PGA) where sputum eosinophils and neutrophils were within the normal range.

The role of non-eosinophilic asthma in children is less clear. Airway inflammation with both eosinophilic and neutrophilic inflammation was reported as a feature of exacerbations in children with asthma (162), but heterogeneity of the inflammatory response in stable childhood asthma is less well understood. According to Payne and
Bush, most children with asthma were well controlled by low or moderate doses of inhaled corticosteroids (197). However, there was a group of older children who have severe asthma and poor response to ICS (197, 198), which were associated with persistent eosinophilic inflammation, or non-eosinophilic inflammation, or persistent airflow limitation (197). Additionally, the clinical features of non-eosinophilic asthma in children are not described.

**Aims**

The aim of this study was to investigate the airway inflammatory phenotypes in children with asthma. I have also compared the clinical characteristics between the different inflammatory phenotypes in childhood asthma.

Further, I sought to identify markers that would allow prediction of inflammatory phenotype by clinical and physiological assessments

**Hypotheses**

I hypothesized that a non-eosinophilic pattern would be present in all grades of childhood asthma.

In addition, I suggested that the different inflammatory phenotypes of childhood asthma would demonstrate similar clinical features. I tested these hypotheses by assessing the distribution of airway phenotypes in children with asthma, and comparing the clinical features between the different inflammatory phenotypes.
An increase in FeNO levels in combination with the occurrence of asthma characteristics may predict an eosinophilic asthma in children.

Methods

Study design

This study used a longitudinal design. Children with asthma were invited to attend between two and three study visits. Visits 2 and 3 were conducted three months and six months after the initial visit, respectively. Children with asthma were required to withhold short acting $\beta_2$-agonist at least 6 hours and long acting-$\beta_2$ agonist for 12 hours prior to their appointments.

All children were required to perform fractional exhaled nitric oxide (FeNO) measurement, exhaled breath condensate (EBC), spirometry, hypertonic saline challenge and sputum induction at each visit. An asthma questionnaire and skin prick test were performed at visit one.

Subjects

Children with asthma were recruited from the Outpatient clinics at John Hunter Children’s Hospital (Newcastle, Australia) and by advertisement. All children were aged between 7-17 years. The children who were studied longitudinally had in fact been taken from the original cross sectional study described in Chapter I.
Children with asthma were previously diagnosed by pediatricians based on clinical and lung function criteria. The change in FEV$_1$ after bronchodilator use was performed at visit 2 and visit 3. Each child’s asthma was classified using the criteria from National Asthma Council Australia (156).

Stable asthma in children was assessed before the techniques were performed at each visit. Children with stable asthma were defined as having no increase in asthmatic symptoms or asthma medication use, no treatment with oral corticosteroid, no unscheduled visit to a GP or hospital due to asthma worsening in the preceding 4 weeks.

**Measurements**

**Questionnaires**

The asthma symptoms and relevant history of children were assessed using a questionnaire from a previous long term cohort study in the Hunter region (the RIFYL study) (178). Parents of children with asthma and children were asked a series of questions to assess the stability of asthma in the preceding 4 weeks. Asthma control was assessed using the Juniper asthma control questionnaire (403).

**Nitric Oxide Measurement**

Measurements of fractional exhaled nitric oxide were made using the single breath online technique according to American Thoracic Society guidelines (412), as described in Methods chapter.
Skin prick testing
Allergen sensitization to four common allergens, *Dermatophagoides pteronyssinus* (DP), *Alternaria tenuis*, cockroach mix and mixed grass was determined by skin prick testing. The positive control was histamine HCL (10mg/ml) and the negative control was a normal saline/glycerin solution (50% v/v glycerin). Atopy was determined as the presence of at least one positive skin reaction.

Exhaled breath condensate
Children were asked to breathe normally with tidal breathing for 10 minutes into a mouthpiece attached to a cooling system. As the children exhaled, the air was cooled and condensed (441). EBC samples were collected and then EBC pH was measured immediately without deaeration.

Spirometry
Spirometry was performed using an electronic spirometer according to ATS guidelines (424). The predicted lung function values of Hibbert *et al* were used as reference values (425).

Saline challenge and sputum induction
A combination of hypertonic saline challenge and sputum induction to assess AHR and airway inflammation in a single test at the same time was performed after spirometry (178). The procedure of saline challenge and sputum induction has been described in Methods chapter.
Induced sputum analysis

Sputum was collected in a sterile container during the bronchial provocation challenge and sputum induction. Sputum portions were selected from saliva and processed as described (178, 426). After processing, a differential cell count was obtained from 400 non-squamous cells counted in a cytospin preparation. The cells were identified by their morphology and expressed as a percent of the total non-squamous cells counted. Eosinophils were determined from slides stained with Chromotrope 2R in the same fashion.

IL-8 measurement

The measurement of IL-8 in sputum supernatant was assessed using the Human DuoSet ELISA Kit (IL-8 ELISA; R & D Systems, Inc. Minneapolis MN, USA) with a standard curve range from 31.2pg/mL to 2000pg/mL.

TNF-α measurement

Measurement of TNF-α in ultracentrifuge sputum sample was assessed using the Human DuoSet ELISA Development Kit for TNF-α Cat no: DY 210 (R & D Systems, Inc. Minneapolis MN, USA) with a standard curve range from 31.3pg/mL to 1000pg/mL.

Classification of asthma phenotypes

Asthma phenotypes were categorized based upon sputum results (161). Children with sputum eosinophils greater than 2.5% were classified as Eosinophilic asthma (EA), children with sputum neutrophils greater than 61% were classified as Neutrophilic
asthma (NA), children with sputum eosinophils less than or equal 2.5% and sputum neutrophils less than or equal 61% were classified as Paucigranulocytic asthma (PGA), and subjects with sputum eosinophils greater than 2.5% and sputum neutrophils greater than 61% were classified as mixed granulocytic asthma (MGA).

Children were required to demonstrate the same inflammatory phenotype based on the induced sputum pattern of airway inflammation on at least two occasions over three visits. The interval between visits was 3 months.

Statistical analysis

Statistical analysis was carried out using STATA (STATA Corporation, College Station, Texas, USA). Characteristics of the study population and parametric results were expressed as geometric mean and standard deviation (SD). Non parametric results were reported as median and interquartile range (IQR). Comparison of clinical features and airway inflammatory markers between different phenotypes of asthma was conducted by unpaired t-test for variables with normal distribution, and nonparametric data was analyzed by Mann-Whitney test. Categorical data was analyzed by Chi- squared test or Fisher's exact test. A p < 0.05 was accepted as statistically significant. The sensitivity, specificity and Youden’s index of clinical assessments were calculated in order to estimate the cut- point for prediction of inflammatory phenotype in children with asthma.
Results

*Inflammatory phenotypes*

There were 35 children with asthma in whom sputum samples were obtained on at least two visits. Thirteen (37.1%) children had a stable eosinophilic asthma phenotype, 16 (45.7%) children had a stable paucigranulocytic phenotype, 1 (2.9%) child had a stable neutrophilic asthma and 5 (14.3%) children changed their airway phenotype between visits. In children with an unstable airway inflammatory phenotype, one subject changed from NA to PGA and four subjects changed from EA to PGA. None of the children had stable mixed granulocytic asthma. The results suggested that EA and PGA were principal phenotypes of airway inflammation in childhood asthma.

*Figure 3.2. 1: The stability of sputum eosinophils and neutrophils in children with eosinophilic pattern. Individual data with median (bar) are displayed.*
The stability of sputum eosinophils and neutrophils in children with eosinophilic asthma demonstrated no difference in either sputum eosinophils or sputum neutrophils between study visits (p=0.2, p=0.95; respectively; Figure 3.2.1). In children with paucigranulocytic asthma, sputum eosinophils were equal to or less than 2.5% and sputum neutrophils were less than 61% at both initial and later visits (p=0.67, p=0.97; respectively; Figure 3.2.2).
Clinical characteristics

Table 3.2.1: Demographic characteristics of children with eosinophilic and paucigranulocytic asthma

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EA (n=13)</th>
<th>PGA (n=16)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean, SD; years)</td>
<td>11.3 ± 3.3</td>
<td>11.1 ± 3.2</td>
<td>0.84 *</td>
</tr>
<tr>
<td>Gender (%male)</td>
<td>53.8</td>
<td>68.8</td>
<td>0.4 #</td>
</tr>
<tr>
<td>Height (mean, SD; cm)</td>
<td>147.5 ± 12.6</td>
<td>151.2 ± 16.6</td>
<td>0.5 *</td>
</tr>
<tr>
<td>Weight (mean, SD; kg)</td>
<td>45.9 ± 12.6</td>
<td>48.9 ± 20.6</td>
<td>0.44 *</td>
</tr>
<tr>
<td>BMI (Body Mass Index) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24.9</td>
<td>84.6</td>
<td>75</td>
<td>0.4 $</td>
</tr>
<tr>
<td>≥ 25</td>
<td>15.4</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard deviation
* = Unpaired t test
# = Pearson Chi$^2$ Test
$ = Fisher’s exact test

The demographic characteristics were similar between children with EA and children with PGA (Table 3.2.1). There tended to be more boys in both asthma groups, consistent with the male predominance of asthma among children.
Table 3.2. 2: Clinical characteristics of children with eosinophilic and paucigranulocytic asthma over the previous 12 months

<table>
<thead>
<tr>
<th>Clinical characteristics over past 12 months (% Subjects)</th>
<th>EA (n=13)</th>
<th>PGA (n=16)</th>
<th>P value $^S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheezing</td>
<td>100</td>
<td>56.25</td>
<td>0.007</td>
</tr>
<tr>
<td>Wheezing during exercise</td>
<td>100</td>
<td>56.25</td>
<td>0.007</td>
</tr>
<tr>
<td>Breathlessness</td>
<td>84.6</td>
<td>62.5</td>
<td>0.18</td>
</tr>
<tr>
<td>Breathlessness at night</td>
<td>69.2</td>
<td>31.25</td>
<td>0.048</td>
</tr>
<tr>
<td>Dry cough at night</td>
<td>84.6</td>
<td>68.75</td>
<td>0.29</td>
</tr>
<tr>
<td>Wakening by asthma symptoms</td>
<td>76.9</td>
<td>50</td>
<td>0.14</td>
</tr>
<tr>
<td>Chest cold</td>
<td>92.3</td>
<td>81.25</td>
<td>0.38</td>
</tr>
<tr>
<td>Asthma attacks</td>
<td>84.6</td>
<td>56.25</td>
<td>0.1</td>
</tr>
<tr>
<td>Visit GP due to asthma attacks</td>
<td>46.2</td>
<td>37.5</td>
<td>0.46</td>
</tr>
<tr>
<td>Absences from school due to asthma</td>
<td>69.2</td>
<td>75</td>
<td>0.53</td>
</tr>
</tbody>
</table>

$^S$= Fisher’s Exact Test

Children with EA reported more asthma symptoms over the previous 12 months than children with PGA (Table 3.2.2). The percentage of childhood asthma with wheeze in the past 12 months in children with EA was 100% compared to 56.25% in children with PGA (p=0.007). The prevalence of breathlessness at night was also higher in children with an eosinophilic pattern compared to children with paucigranulocytic pattern (69.2% versus 31.25%, p=0.048). Asthma exacerbations, as assessed by visits to GP due to asthma attacks, absence from school due to asthma in the past 12 months, and the frequency of asthma attacks in the last 12 months, were similar in children with EA and children with PGA (p= 0.46, 0.53, 0.1; respectively). In summary, asthma symptoms seemed to be more severe in the children with an eosinophilic pattern compared to the children with a
paucigranulocytic pattern, but both EA and PGA groups experienced asthma exacerbations to a similar degree.

*Table 3.2.3: History of children with asthma*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EA (n=13)</th>
<th>PGA (n=16)</th>
<th>P value $ \text{^3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Personal history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eczema</td>
<td>69.2</td>
<td>25</td>
<td>0.022</td>
</tr>
<tr>
<td>Gastro-oesophageal reflux</td>
<td>7.7</td>
<td>6.25</td>
<td>0.7</td>
</tr>
<tr>
<td>Allergic Rhinitis</td>
<td>69.2</td>
<td>62.5</td>
<td>0.51</td>
</tr>
<tr>
<td>Allergies</td>
<td>69.2</td>
<td>25</td>
<td>0.022</td>
</tr>
<tr>
<td><strong>Maternal history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>38.5</td>
<td>37.5</td>
<td>0.96</td>
</tr>
<tr>
<td>Hay fever</td>
<td>69.2</td>
<td>37.5</td>
<td>0.09</td>
</tr>
<tr>
<td>Eczema</td>
<td>23.1</td>
<td>37.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Allergy</td>
<td>30.8</td>
<td>56.25</td>
<td>0.16</td>
</tr>
<tr>
<td>Chest diseases</td>
<td>7.7</td>
<td>12.5</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Sibling’s diseases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>41.7</td>
<td>33.3</td>
<td>0.66</td>
</tr>
<tr>
<td>Eczema</td>
<td>41.7</td>
<td>6.7</td>
<td>0.043</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>41.7</td>
<td>26.7</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Living in the same house with smokers when the child was a baby</strong></td>
<td>30.8</td>
<td>43.75</td>
<td>0.37</td>
</tr>
</tbody>
</table>

$\text{^3}= \text{Fisher’s Exact Test}$
The history of children with asthma is reported in table 3.2.3. The frequencies of eczema and allergies were significantly higher in children with eosinophilic pattern compared to children with paucigranulocytic pattern (69.2% versus 25%, \( p=0.022 \); 69.2% versus 25%; \( p=0.022 \); respectively). However, the frequencies of gastro-esophageal reflux and allergic rhinitis were similar between children with EA and children with PGA (\( p=0.7 \); 0.51; respectively).

There was no difference in maternal history of diseases such as asthma (\( p=0.96 \)), eczema (\( p=0.47 \)), allergy (\( p=0.16 \)), and chest diseases (\( p=0.56 \)) between children with EA and children with PGA. The frequency of hay fever tended to be lower in mothers of children with PGA compared to mothers of children with EA (37.5% versus 69.2%, \( p=0.09 \)).

The prevalence of eczema in the siblings was significantly higher in children with EA compared to children with PGA (41.7% versus 6.7%, \( p=0.043 \)). However, the prevalence of asthma and allergic rhinitis in the siblings of children with EA and children with PGA were similar (\( p=0.66 \), 0.34; respectively).

The prevalence of children living with household smokers when a child was a baby was the same between children with EA and PGA (30.8% versus 43.75%; \( p=0.37 \)).
Table 3.2.4: Asthma control in the past 1 week in children with eosinophilic asthma and paucigranulocytic asthma (median, IQR)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EA (n=13)</th>
<th>PGA (n=16)</th>
<th>P value +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woken by asthma</td>
<td>0 ( 0-0)</td>
<td>0 (0-0)</td>
<td>0.43</td>
</tr>
<tr>
<td>Symptoms upon waking</td>
<td>1 (0-1)</td>
<td>0 (0-0)</td>
<td>0.005</td>
</tr>
<tr>
<td>Activities limited by asthma</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0.5</td>
</tr>
<tr>
<td>Breathlessness due to asthma</td>
<td>1 (0-2)</td>
<td>0 (0-1)</td>
<td>0.13</td>
</tr>
<tr>
<td>Wheeze</td>
<td>1 ( 1-2)</td>
<td>0 ( 0-0.5)</td>
<td>0.003</td>
</tr>
<tr>
<td>SABA puffs per day</td>
<td>1 (0-2)</td>
<td>0 (0-1)</td>
<td>0.025</td>
</tr>
<tr>
<td>FEV1% predicted</td>
<td>2 (0-2)</td>
<td>1 (0-2)</td>
<td>0.085</td>
</tr>
<tr>
<td>Overall score</td>
<td>1 (0.57-1.29)</td>
<td>0.29 (0.14-0.49)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

SABA: short acting β2 agonists
+ = Mann-Whitney Test

Asthma control was assessed, based upon asthma symptoms, lung function and short-acting β agonist use in the past 1 week (403) (Table 3.2.4). The median (IQR) asthma control score in EA was 1 (0.57-1.29), which was significantly higher than in PGA [0.29 (0.14-0.49); p=0.009]. A comparison of the individual asthma control items between the two groups showed that in EA there were increased asthma symptoms when the child woke up in the morning (p=0.005), increased frequency of wheeze in the past week (p=0.003), and increased daily short acting β2 agonist use (p=0.025) compared to children of PGA. There were no differences in the frequency of night waking from asthma, limited activity due to asthma and shortness of breath due to asthma between the two groups.
FEV₁% predicted tended to be higher in children with PGA (p=0.085). Children with a PGA phenotype had better controlled asthma than children with the EA phenotype.

*Figure 3.2.3: Asthma triggers in children with eosinophilic asthma (EA) and paucigranulocytic asthma (PGA). *p <0.05*

The triggers that induced asthma symptoms were also different between EA and PGA (Figure 3.2.3). Children with increased sputum eosinophilia were more likely to report asthma that was triggered by viral infection and allergen exposure than children with PGA (100% versus 68.75%, p=0.037; 100% versus 56.25%, p=0.007; respectively).
Table 3.2.5: Clinical asthma pattern between PGA and EA group

| Characteristics (%) Subjects | EA (n=13) | PGA (n=16) | P value $^5$
|-----------------------------|----------|------------|----------------
| Infrequent episodic         | 15.4     | 37.5       | 0.3            |
| Frequent episodic           | 23.1     | 50         | 0.14           |
| Persistent                  | 61.5     | 12.5       | 0.008          |

Overall score: 0.029

$^5$ = Fisher’s Exact Test

The clinical pattern of children with asthma was assessed based upon asthma symptoms and lung function as described in the Australian NAC guideline (156) (Table 3.2.5). Children with eosinophilic asthma had a more persistent asthma pattern than children with PGA (61.5% versus 12.5%; p=0.008). Overall, children with EA had more severe asthma than children with PGA (p=0.029).

Table 3.2.6: Asthma treatment between children with EA and PGA

| Characteristics ( % subjects) | EA (n=13) | PGA (n=16) | P value $^5$
|-------------------------------|----------|------------|----------------
| ICS used                     | 84.6     | 43.75      | 0.029 $^5$
| ICS dose ( median, IQR ; μg/daily) | 500 (250-1000) | 0 (0-500) | 0.03 $^+$
| Montelukast ( % subjects)    | 7.7      | 6.25       | 0.7 $^5$

$^5$ = Fisher’s Exact Test
$^+$ = Mann-Whitney Test
Overall, 63.6% of children with asthma were taking ICS and 6.6% were using montelukast for maintenance asthma therapy. Children with EA reported more daily use of ICS compared to children with PGA (84.6% versus 43.75%, p=0.029) (Table 3.2.6). Similarly, the daily dose of ICS used by the EA group was higher than that of the PGA group (500μg versus 0μg, p=0.03). No difference in montelukast use between the two groups was observed (7.7% versus 6.25% p=0.7).

**Objective measurements**

**Atopy**

*Table 3.2.7: Atopic status in children with eosinophilic and paucigranulocytic asthma*

<table>
<thead>
<tr>
<th>Characteristics ( % subjects)</th>
<th>EA (n=13)</th>
<th>PGA (n=16)</th>
<th>P value $^\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopy</td>
<td>100</td>
<td>62.5</td>
<td>0.017</td>
</tr>
<tr>
<td>Alternaria</td>
<td>53.8</td>
<td>31.25</td>
<td>0.22</td>
</tr>
<tr>
<td>Dust mite</td>
<td>100</td>
<td>56.25</td>
<td>0.007</td>
</tr>
<tr>
<td>Cockroach</td>
<td>46.2</td>
<td>12.5</td>
<td>0.055</td>
</tr>
<tr>
<td>Grass mix</td>
<td>69.2</td>
<td>43.75</td>
<td>0.17</td>
</tr>
</tbody>
</table>

$^\dagger$= Fisher’s Exact Test

All of the subjects with sputum eosinophilia were sensitized to at least one of the allergens tested, whereas 62.5% of paucigranulocytic asthma were atopic (p=0.017; Table 3.2.7).
3.2.7). Similar results were found for the prevalence of house dust mite sensitization (100% versus 56.25%; \( p=0.007 \)).

The proportion of *Alternaria* sensitization, Cockroach sensitization, Grass mix sensitization were higher in children with EA compared to children with PGA, but they failed to reach statistical significance (\( p=0.22, 0.055, 0.17 \); respectively).

**Lung function**

*Table 3.2.8: Lung function of children with eosinophilic and paucigranulocytic asthma*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EA (n=13)</th>
<th>PGA (n=16)</th>
<th>( P ) value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV(_1)% predicted (mean, SD)</td>
<td>86.6 ± 14.9</td>
<td>94.2 ± 10.9</td>
<td>0.12</td>
</tr>
<tr>
<td>% FEV(_1)/FVC (mean, SD)</td>
<td>74.6 ± 8.8</td>
<td>83.75 ± 6.7</td>
<td>0.004</td>
</tr>
</tbody>
</table>

SD: Standard deviation  
* = Unpaired t test

The mean FEV\(_1\) % predicted (SD) was 94.2% (10.9%) for children with PGA and 86.6% (14.9%) for children with EA. The FEV\(_1\) in children with PGA tended to be higher than in children with EA (\( p=0.12 \)). The % FEV\(_1\)/FVC ratio in children with eosinophilic asthma was significantly lower compared to children with paucigranulocytic asthma (74.6% versus 83.75%, \( p=0.004 \)). Children with EA had more severe airflow obstruction than children with PGA (Table 3.2.8).
Airway hyperresponsiveness

Table 3.2.9: Comparison in AHR between children with eosinophilic and paucigranulocytic asthma

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EA (n=13)</th>
<th>PGA (n=16)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHR (%)</td>
<td>84.6</td>
<td>43.75</td>
<td>0.03 $</td>
</tr>
<tr>
<td>PD15 (mL)</td>
<td>2.04 (1.03-4)</td>
<td>9.44 (4.83-15.98)</td>
<td>0.005 $</td>
</tr>
</tbody>
</table>

$ = Fisher’s Exact Test  
+ = Mann-Whitney Test

Airway hyperresponsiveness was more frequent in children with eosinophilic pattern than children with paucigranulocytic pattern (84.6% versus 43.75%, p=0.03). In addition, AHR was also more severe in children with EA compared to PGA (p=0.005, Table 3.2.9).
Fractional exhaled nitric oxide

*Figure 3.2.4: The median of fractional exhaled Nitric Oxide in children with eosinophilic asthma (EA) and paucigranulocytic asthma (PGA)*

The median FeNO levels (IQR) were 45.66 (39.5- 57.1) ppb for children with EA and 16.5 (9.1-28) ppb for children with PGA (Figure 3.2.4). The FeNO levels in eosinophilic asthma was significantly increased compared to paucigranulocytic asthma (p=0.0034).

Exhaled breath condensate

*Table 3.2.10: pH of EBC in children with eosinophilic and paucigranulocytic asthma*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EA (n=13)</th>
<th>PGA (n=16)</th>
<th>P value *+</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (median, IQR)</td>
<td>7.2 (6.9-7.5)</td>
<td>7.4 (6.8-7.8)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

*+ = Mann-Whitney Test
There was no difference in EBC pH between children with eosinophilic pattern and children with paucigranulocytic pattern (7.2 versus 7.4, p=0.95; Table 3.2.10).
Induced sputum cell counts

*Table 3.2.11: Induced sputum cell counts in children with eosinophilic asthma (EA) and paucigranulocytic asthma (PGA).*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EA (n=13)</th>
<th>PGA (n=16)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality, median (IQR)</td>
<td>16 (13-18)</td>
<td>18 (17-20)</td>
<td>0.025</td>
</tr>
<tr>
<td>Viability % median (IQR)</td>
<td>67.39 (19.4-79.3)</td>
<td>73.7 (66.6-89)</td>
<td>0.09</td>
</tr>
<tr>
<td>TCC x 10⁶/ml median (IQR)</td>
<td>2.16 (1.71-4.14)</td>
<td>3.15 (1.53-9.99)</td>
<td>0.75</td>
</tr>
<tr>
<td>Neutrophils % median (IQR)</td>
<td>16.38 (5.75-33.75)</td>
<td>8 (5.75-17.5)</td>
<td>0.38</td>
</tr>
<tr>
<td>Neutrophils absolute x10⁴/ml, median (IQR)</td>
<td>27 (5.58-126.27)</td>
<td>23 (11.12-223.02)</td>
<td>0.64</td>
</tr>
<tr>
<td>Eosinophils % median (IQR)</td>
<td>18 (9.9-28.5)</td>
<td>0.25 (0-1.25)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Eosinophil absolute x10⁴/ml, median (IQR)</td>
<td>35.1 (12.44-70.2)</td>
<td>0.38 (0-4.25)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Macrophage % median (IQR)</td>
<td>56 (44.5-67.5)</td>
<td>83.25 (70.5-91.25)</td>
<td>0.004</td>
</tr>
<tr>
<td>Macrophage absolute x10⁴/m, median (IQR)</td>
<td>156.74 (77.76-242.2)</td>
<td>298.86 (127.9-543.6)</td>
<td>0.065</td>
</tr>
<tr>
<td>Lymphocyte % median (IQR)</td>
<td>1 (0.63-1.88)</td>
<td>1.2 (0.5-2.25)</td>
<td>0.98</td>
</tr>
<tr>
<td>Epithelial % median (IQR)</td>
<td>1.1 (0.13-2.63)</td>
<td>2.25 (0.5-4.25)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* = Mann-Whitney Test
The quality of sputum samples was higher in a paucigranulocytic group compared with an eosinophilic group (p=0.025). Children with an eosinophilic asthma had increased % sputum eosinophils and absolute sputum eosinophils compared to children with paucigranulocytic asthma (p= 0.0002, p=0.0006; respectively; Table 3.2.11). There were no differences in total cell count (TCC), % sputum neutrophils, absolute sputum neutrophils, % sputum lymphocyte and % sputum epithelial cells between children with eosinophilic asthma and children with paucigranulocytic asthma (p>0.05). The percentage of sputum macrophages was 56% for children with EA and 83.25% for paucigranulocytic group (p=0.003). The absolute sputum macrophages tended to increase in children with PGA compared to children with EA (p=0.065, Table 3.2.11).

Sputum cytokines

Table 3.2.12: Comparison in sputum IL-8 between children with asthma with the different airway phenotypes

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EA (n=11)</th>
<th>PGA (n=15)</th>
<th>P value +</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 (ng/ml)</td>
<td>1.67 (1.14-15.71)</td>
<td>1.54 (0.89-3.29)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

+ = Mann-Whitney Test

The median IL-8 (IQR) in children with EA was 1.67 ng/ml (1.14-15.71) whereas it was 1.54 ng/ml (0.89-3.29) in children with PGA. There was not significantly different in sputum IL-8 between children with EA and children with PGA (p=0.35).
The ability of tests to detect eosinophilic pattern in children with asthma

This study found that there were differences in clinical features, lung function, AHR, FeNO levels, and atopic status between children with EA and children with PGA. The ability of clinical markers to predict eosinophilic asthma was assessed (Table 3.2.13).

Table 3.2.13: Operating characteristics of tests for eosinophilic asthma in children

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Youden’s Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeNO &gt;25 ppb</td>
<td>84.6%</td>
<td>68.8%</td>
<td>0.53</td>
</tr>
<tr>
<td>FeNO &gt;35 ppb</td>
<td>84.6%</td>
<td>87.5%</td>
<td>0.72</td>
</tr>
<tr>
<td>FeNO &gt; 45 ppb</td>
<td>53.8%</td>
<td>87.5%</td>
<td>0.41</td>
</tr>
<tr>
<td>Atopy</td>
<td>100%</td>
<td>37.5%</td>
<td>0.38</td>
</tr>
<tr>
<td>Atopy + FeNO &gt;25 ppb</td>
<td>100%</td>
<td>75%</td>
<td>0.75</td>
</tr>
<tr>
<td>ACS &gt;0.5</td>
<td>84.6%</td>
<td>75%</td>
<td>0.6</td>
</tr>
<tr>
<td>ACS &gt; 0.5 + FeNO &gt;25 ppb</td>
<td>90.9%</td>
<td>81.8%</td>
<td>0.73</td>
</tr>
<tr>
<td>FEV₁/FVC &lt; 80%</td>
<td>69.2%</td>
<td>75%</td>
<td>0.44</td>
</tr>
<tr>
<td>FEV₁/FVC &lt; 80% + FeNO &gt;25 ppb</td>
<td>100%</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>AHR ( PD₁₅ &lt; 16)</td>
<td>84.6%</td>
<td>56.3%</td>
<td>0.41</td>
</tr>
<tr>
<td>AHR + FeNO &gt;25 ppb</td>
<td>90.9%</td>
<td>77.8%</td>
<td>0.69</td>
</tr>
<tr>
<td>DRS &gt;3</td>
<td>83.3%</td>
<td>87.5%</td>
<td>0.71</td>
</tr>
<tr>
<td>DRS &gt;3 + FeNO &gt;25 ppb</td>
<td>100%</td>
<td>90.9%</td>
<td>0.91</td>
</tr>
</tbody>
</table>

ACS : Asthma control score
AHR : Airway hyperresponsiveness
PD₁₅ : Provocative dose of hypertonic saline causes to fall FEV₁ by 15% of the baseline.
DRS : Dose response slope
The Youden’s index is defined as sensitivity + specificity - 1, where sensitivity and specificity are calculated as proportions.

Dose-response slope (DRS) reflects the fall in FEV1 per unit substance inhaled. In this study, the DRS was assessed by dividing the % fall in FEV1 from pre-challenge FEV1 by the amount of hypertonic saline inhaled.

An elevated FeNO (>25ppb) had a sensitivity of 84.6% and a specificity of 68.8% for eosinophilic asthma in this group of children. Sensitivity was reduced (53.8%) when a higher cut-point of 45 ppb was used. Atopic sensitization had a high sensitivity for EA (100%) but specificity was low (37.5%). The combination of atopic sensitization and elevated FeNO levels (25 ppb) improved specificity for the detection of EA to 75%. The median asthma control score of children with asthma in this study was 0.5. With an asthma control score more than 0.5, the sensitivity was 84.6% and the specificity was 75% for eosinophilic asthma. When a combination of asthma control score and elevated FeNO levels was used, both sensitivity and specificity were increased (90.9%, 81.8%, respectively). An FEV1/FVC ratio less than 80% had a sensitivity of 69.2% and a specificity of 75% for EA. Both the sensitivity and specificity for detection to EA were 100% when FEV1/FVC ratio was used in combination with elevated FeNO levels (>25pp). AHR to 4.5% saline had a sensitivity of 84.6% and specificity of 56.3% for detection to EA. These values were improved by the addition of FeNO >25 ppb to AHR, where sensitivity rose to 90.9% and specificity improved to 77.8%. AHR can also be expressed as the dose response slope (DRS). The combination of elevated FeNO (>25 ppb) and DRS (≥ 3) had the second highest combination of sensitivity (100%) and
specificity (90.9%) to detect EA. In summary, the combination of elevated FeNO levels (>25ppb) and DRS (≥ 3) or FEV<sub>1</sub>/FVC ratio (<80%) had the best performance characteristics for the detection of eosinophilic asthma.

**Discussion**

This study found that children with stable asthma exhibit two main inflammatory phenotypes, one with increased sputum eosinophils and another with normal numbers of sputum eosinophils and neutrophils. Children with an eosinophilic asthma had more severe asthma, with increased asthma symptoms, airway obstruction, increases the frequency and the severity of AHR, and increased atopic status compared to children with paucigranulocytic asthma. Pathological assessment found that children with an eosinophilic pattern had higher FeNO, elevated sputum eosinophils and decreased sputum macrophages compared to children with a paucigranulocytic pattern. An increase in FeNO level in combination with other features of asthma such as atopy, high asthma control score, low FEV<sub>1</sub>/FVC ratio, or AHR would predict eosinophilic asthma.

1- Airway phenotypes

While increased eosinophils are accepted as a pathological characteristic of asthma, this study found that children with asthma without increased sputum eosinophils were common. The results of our study are similar to those of Douwes, who found that approximately 50% of asthma had eosinophilic airway inflammation (160). Gibson *et al* investigated airway inflammation in 56 adults with persistent asthma, and a non-eosinophilic pattern of inflammation was reported in 59% (159).
Paucigranulocytic asthma

This study found that children with asthma with a paucigranulocytic pattern were common. This group had similar sputum features to healthy children, with neither increased sputum eosinophils nor increased sputum neutrophils. Interestingly, subjects with PGA showed a higher proportion of sputum macrophages compared to subjects with EA. Although they had no or little change in airway inflammation compared to healthy children, children with PGA showed typical clinical asthma symptoms such as wheeze, breathlessness, and nocturnal cough but these symptoms were less severe compared to asthmatic subjects with increased sputum eosinophils. The paucigranulocytic pattern existed in all severity grades of asthma. Gibson et al investigated the increased frequency of asthma in adolescents who migrated to Australia and found that the development of wheeze after migration to Australia was not related to atopy, AHR, or eosinophilic airway inflammation but related to non-eosinophilic asthma mechanisms (503). A possible mechanistic explanation for PGA is that clinical asthma was driven by macrophage activation, or other mechanisms such as neurogenic mechanisms.

Currently, there is little data that has investigated the correlation between airway macrophages and clinical features of asthma. Macrophages are known to synthesize several growth factors including GM-CSF, PDGF, and several chemokines that promote the proliferation and survival of inflammatory cells (504). In contrast, macrophages release other cytokines such as IL-10, TGF-β that inhibit inflammation (505, 506), but can promote AHR (507). The higher sputum macrophages in both healthy children and childhood asthma with a paucigranulocytic pattern compared to childhood eosinophilic
asthma raises the question of whether macrophages may have a role in the inhibition of eosinophilic airway inflammation in asthma. Further investigations should be carried out to assess the role of macrophages in airway inflammation.

**Neutrophilic asthma**

In contrast to adult asthma, neutrophilic asthma was very uncommon in the children who participated in this study. Neutrophilic asthma has a prevalence of about 20% in stable adult asthma (161). Ronchi et al studied 43 stable subjects with mild or moderate asthma and found that 28% of subjects showed increased sputum neutrophils (508). Both increased sputum neutrophil percentages and absolute neutrophil counts were observed in adults with stable persistent asthma (159). Jatakanon et al demonstrated that sputum revealed significantly increased neutrophil numbers in adults with severe persistent asthma (193). Similarly, Wenzel et al found that neutrophilic asthma in adults was associated with severe asthma (189), suggesting a role for neutrophils in the inflammatory process in adults with stable asthma. Studies also report that severe and refractory asthmatic patients often respond poorly to corticosteroids. Investigations of airway inflammation in those subjects demonstrate suppressed eosinophils, and increased neutrophils (189). Neutrophil percentage, absolute neutrophil counts and IL-8 were increased in subjects with non-eosinophilic asthma (159). Higher levels of neutrophils have been identified from sputum (193) and bronchial wash specimens in severe asthma (189), and a higher concentration of neutrophils has been observed in BAL fluid from patients with severe asthma compared with mild-moderate asthma (194). Interestingly, a neutrophilic pattern was also observed in mild or moderate asthma (160), as well as
acute (162, 191) and stable asthma (500). The findings suggest that neutrophils may have an important role in the pathophysiology of asthma in adults. In summary, neutrophilic asthma is found in all grades of stable adult asthma but it is often correlated with severe persistent asthma.

In children, neutrophilic inflammation often occurs in asthma exacerbations. Systemic neutrophil activation was observed in acute preschool viral wheeze (196). Airway inflammation with both eosinophilic and neutrophilic inflammation was a characteristic feature of exacerbations in children with asthma (162). A proportion of neutrophils in BAL was related to the severity of disease (174). Lex et al investigated airway inflammation in difficult childhood asthma, which was defined as persistent asthma symptoms despite using maximal ICS dose, and found that about 10% of this group had high sputum neutrophils (≥54%) (509). Bush suggested that there was a different phenotype of airway inflammation in older children who had more severe asthma and a poor response to high dose of ICS (198), that may be correlated with neutrophilic asthma. This study observed only one 17 year old child (2.9%) with NA. Only about 5% of children with asthma have persistent asthma (156), which may explain the low prevalence of a neutrophilic pattern in stable childhood asthma. The findings suggested that there are different phenotypes of airway inflammation in childhood and adult asthma.

Several factors are associated with neutrophilic airway inflammation in asthma. A wide range of environmental factors including exposure to tobacco smoke (348), occupational sensitizers (510, 511), air pollutants (512), bacterial infection (513) as well as the
presence of fixed airflow obstruction (514) may contribute to the development of
neutrophilic asthma. In addition, the yearly fall in FEV₁ was negatively correlated with %
sputum neutrophils (515), suggesting that a longer duration of asthma maybe associated
with neutrophilic asthma. Little et al also reported that the duration of asthma was
associated with a higher percentage of sputum neutrophils (516). Children with mild
asthma who are subjected to cigarette smoke could either have a mild form of chronic
bronchitis secondary to smoke exposure or an increased tendency to respiratory tract
infections resulting in airway neutrophilic pattern. Each of these factors is less common
in children, which may explain why NA is uncommon in children compared to adults
with asthma.

Eosinophilic asthma

Airway eosinophilia has long been associated with asthma in both children and adults. In
stable asthma, sputum analysis showed an increase in eosinophils, but not neutrophils
(426, 491). Airway eosinophilia was already present in children with mild asthma (181).
Sputum eosinophils in symptomatic asthma were increased compared to asymptomatic
asthmatic children (517). A study in children with atopic asthma found that airway
inflammation was still ongoing during asymptomatic periods, as demonstrated by
eosinophil and mast cell recruitment, but not in children with viral associated wheeze or
atopy alone (173). Sputum eosinophilia was suggested as a marker for asthma severity
and a positive response to corticosteroid in asthma (179, 518, 519). The current study
supports these results, but also demonstrates that airway inflammation in childhood
asthma involves a heterogeneous process of which sputum eosinophils is only one part (520).

Previous studies in adults with asthma have found that the clinical features of asthma seemed to be similar across the different airway inflammatory phenotypes (379). Also Simpson et al reported apart from a tendency to more severe AHR, clinical symptoms and lung function were similar across phenotypes (161). The situation seems to be different in childhood asthma. This study found that asthma symptoms were more severe in children with increased sputum eosinophils compared to children without increased sputum eosinophils. Subjects with EA reported a higher frequency of wheeze in the last 12 months, higher prevalence of wheezing during exercise, and more breathlessness at night compared to subjects with PGA. Asthma control over the previous week was also worse in EA compared to PGA. Sputum eosinophils have been reported to correlate with the symptoms of asthma control such as the frequency of nocturnal symptoms or β2 – agonist requirements (518).

Our study assessed the clinical pattern of children with asthma based upon asthma symptoms, the frequency of episodes of asthma and asthma medication used over the last 12 months. The asthma classification was determined according to Australian NAC guidelines (156). Subjects with EA were likely to be classified as more severe asthma compared with subjects with PGA. There were significantly more EA subjects with persistent asthma compared with PGA subjects. In contrast, children who were classified as infrequent episodic asthma tended to have a paucigranulocytic phenotype. This study
looked at children individually classified as EA and PGA and found eosinophils to be related to a more persistent asthma pattern. Gibson et al found similar results when group data were examined (521), supporting a role for sputum eosinophils in more severe forms of the disease.

Although sputum eosinophils were increased in children with asthma compared to healthy controls, whether the increased sputum eosinophils reflect the severity of asthma is still controversial. Wilson et al did not find any correlation between the percentage of sputum eosinophils and a clinical index of asthma severity (199) or current asthma symptoms (459). According to Gibson, the clinical pattern of asthma was related to the degree of airway inflammation, and the increasing inflammatory process was characterized by increased sputum eosinophils. There were a significantly increased airway eosinophils with increasing clinical asthma symptoms (181, 521). Children with persistent asthma had significantly increased sputum eosinophils compared to infrequent episodic asthma (521). Covar et al also found that children with moderate to severe asthma had significantly increased sputum eosinophils than those who had mild asthma (518). Belda et al showed the evidence of higher risk of asthma exacerbation in 12 months in subjects with eosinophilic inflammation (522). Other studies confirmed the increased sputum eosinophil levels were associated with an increase in clinical variables of asthma (178, 523).

Inhaled corticosteroid is an important medication in the management of asthma. ICS use may improve clinical asthma and reduce sputum eosinophils (524). This raises the
possibility that the children with PGA may be EA but sputum eosinophilia has been suppressed by ICS. This seems unlikely because more children with EA than PGA were taking ICS, and children with EA used a higher ICS daily dose than subjects with PGA. The National Asthma Council recommends that 500 μg beclomethasone daily is appropriate for children with asthma (156). Prior observations have also found a relationship between increased ICS treatment intensity and increased sputum eosinophils (521). Cai reported that sputum eosinophils could remain elevated in children with asthma who took high doses of ICS (491). In addition, Wenzel also found the high levels of eosinophils despite the high dose of ICS (189). The presence of sputum eosinophilia in children using ICS may be due to relative steroid resistance or poor adherence.

According to NAC Australia, about 75% of children with asthma were infrequent intermittent asthma and only 5% of childhood asthma suffered from persistent asthma (156). No or little difference in the airway inflammatory cells between healthy children and children with PGA may be explained by the less asthma severity, normal lung function, and lower percentage of AHR in children with PGA compared to children with EA. Because childhood asthma with a paucigranulocytic phenotype was common, that resulted in less asthma severity in children compared to adults. Neutrophilic asthma is associated with more severe asthma, a poor response to corticosteroids, and is not common in children.
2- Asthma triggers

Children with asthma can experience symptoms that are induced by different triggers such as allergens, air pollutants, viruses, etc., which cause inflammation and provoke acute bronchoconstriction. Both activated eosinophils and neutrophils were identified in sputum and BAL in asthma exacerbations which were associated with increased levels of IL-5, IL-8, and of proinflammatory mediators. Inhalation of an allergen in subjects with asthma who were sensitized caused an airway inflammatory response and bronchoconstriction (525). Allergen exposure in atopic asthmatic patients was associated with recruitment and activation of eosinophils in the airways (526). Airway eosinophils were significantly increased after allergen exposure suggesting that allergen can induce eosinophilic inflammation in atopic asthma (526, 527). An experimental study found that mice developed BAL eosinophilia and pulmonary eosinophilia after exposure to cockroach (528). Eosinophils in lung tissue also accumulated to higher levels after challenge with aerosolized ovalbumin in allergic mice (529). Experimental exposure to house dust mite allergens caused bronchoalveolar inflammatory responses, involving the recruitment and activation of inflammatory cells including eosinophils, mast cells, neutrophils, monocytes and lymphocytes (530).

The asthma triggers such as viruses, endotoxin or allergen exposure, were able to recruit neutrophils, via IL-8 production from activated macrophages or epithelial cells (531). Viral infections are common triggers of acute asthma in both children and adults. A prominent neutrophilic inflammation with high levels of IL-8 during the asthma exacerbation is believed to be the response caused in viral induced asthma (162).
Airborne endotoxin can also trigger neutrophic inflammation in asthma because it can recruit neutrophils to the airway and increase the susceptibility to rhinovirus-induced colds (532). Respirable endotoxin (or LPS) has also been reported to induce airway inflammation with increased eosinophils in the airways of subjects with atopic asthma (533).

Exercise induced bronchoconstriction is a common phenomenon, which occurs in about 80% - 90% of subjects with asthma (534). This is generally believed to be due to mediator release. In one study, significant increases in sputum eosinophils were observed in group with exercise-induced asthma after exercise challenge (535). According to Lee, exercise-induced bronchoconstriction may related to eosinophilic inflammation in the airways of children with asthma (536).

Smokers often suffer more acute respiratory tract infections and acute asthma attacks (305, 306). In addition, there is increasing evidence of elevated asthma symptoms and reduced lung function in children with asthma who were exposed to ETS (251, 334). Raherison et al suggested that asthma that developed before smoking was related to atopic status and allergic inflammation (537). Smokers who subsequently develop asthma have non eosinophilic inflammation (316, 348), with increased sputum IL-8 compared to nonsmokers, both in healthy subjects and subjects with asthma (348).

The roles of several environmental exposures that trigger attacks in subjects with asthma have been identified. Outdoor exposure to air pollutants (ozone, nitrogen dioxide, sulphur...
dioxide etc) causes increased exacerbations of asthma (538, 539). Atmospheric pollutants such as ozone exposure can induce non-eosinophilic inflammation of the airways. Significantly increased sputum neutrophil percentage and IL-8 levels after ozone exposure were reported (540, 541), that may explain the increased asthma morbidity associated with ozone pollution episodes observed in epidemiologic studies (541).

Studies have also found that emotional stress triggered asthma exacerbations. Joachim et al reported stress can exacerbate airway hyperreactivity and airway inflammation (by increased BAL eosinophils) in an animal model of allergic bronchial asthma (542). However, the mechanisms of emotional stress that induce acute asthma were still unclear.

In summary, asthma is complex disease which can be triggered by many different triggers. The triggers interact with airway inflammation and may induce short term changes in inflammatory cells. There are no published studies that have investigated the relationship between asthma triggers and pattern of AI. The current study found that children with increased sputum eosinophils were more likely to report their asthma to be triggered by viral infection and allergen exposure than children without increased sputum eosinophils. This could occur if the children had heightened AHR that was induced by EA, making them more susceptible to inflammatory triggers. However, the reliability of trigger reporting by parents or children may be confounded by subjective feelings, resulting in a potential confounder in association between asthma trigger factors and airway inflammatory phenotypes. In addition, asthmatic subjects may also suffer single
or multiple triggers which could alter the pattern of airway inflammation in asthma. Investigating of the pattern of AI in both stable and exacerbation periods in the same subjects with asthma is needed to further understand the relationship between asthma trigger and pattern of AI. Some triggers such as virus, allergen, exercise, air pollutant or ETS exposure induced asthma will be experienced in animals, and even in humans.

3- Atopy

Both children with EA and children with PGA had a high prevalence of atopic sensitizations measured by skin prick testing. However, the group with EA had a significantly higher atopic status compared to group with PGA.

Atopy has been seen as one of the strongest risk factors for the development of asthma (445). A total of 60% of adults and 80% of children with asthma have positive skin-prick tests for environmental allergens (43). Airway inflammation is a feature of both atopic and nonatopic asthma (157). Tsoumakidou investigated airway inflammation in atopic asthmatic adults and found that 54% of subjects had an eosinophilic pattern and 46% had non-eosinophilic asthma (543). My results show similar findings in children, and suggest that atopic status relates to the development of both eosinophilic asthma and non-eosinophilic asthma. The mechanisms of allergen induced eosinophilia are well described, whereas the specific mechanisms of how atopy may contribute to non-eosinophilic asthma are poorly understood (530).
4- Fractional exhaled Nitric oxide

My study found a significant increase in FeNO levels in children with EA compared to children with PGA. This result was similar to a study reported by Payne, in which FeNO levels were only increased in childhood asthma with raised eosinophils (544). An increase in exhaled NO was correlated with the numbers of sputum eosinophils (467, 470) and sputum ECP in children with asthma (467). FeNO levels were also correlated with percentages of eosinophils and ECP in the BAL fluid in childhood asthma (481).

5- Lung function

Children with asthma often have normal lung function values, except during asthma exacerbations. While some studies do not find a significant correlation between sputum eosinophils and FEV$_1$ % predicted (199, 521), other studies indicates an inverse correlation between sputum eosinophils and lung function, as measured by FEV$_1$ (491, 508), and airway obstruction, as measured by FEV$_1$/FVC ratio (508, 518). Ten Brinke suggested that only the presence of sputum eosinophils was independently associated with persistent airway obstruction (OR=8.9; 95%CI: 1.3-59) (545). Turner et al found that subjects without increased eosinophils had less airway obstruction in comparison with subjects with EA (191). Wenzel reported that subjects with increased airway eosinophils had a higher incidence of respiratory failure than those with reduced airway eosinophils (189). In this study, the FEV$_1$ % predicted and FEV$_1$/FVC ratio in children with EA was lower compared to children with PGA. The finding suggests that increased airway eosinophils and increased airway obstruction were characteristics of esinophilic asthma.
6- Airway hyperresponsiveness

AHR is a clinical characteristic of asthma. Sputum eosinophils were related to the degree of AHR in subjects with asthma (178, 508). Lemiere et al found a weak but significant correlation between sputum eosinophils and airway responsiveness (546). Ferguson et al found an association between the numbers of eosinophils in lavage fluid and the degree of AHR in children with asthma (547). Gibson et al reported that sputum eosinophils, but not BAL eosinophils correlated with AHR to hypertonic saline solution (200). Adults with non eosinophilic asthma had less severe AHR compared to subjects with eosinophilic asthma (191). Other studies find a dissociation between the degree of airway responsiveness and the number of eosinophils in sputum (521) or bronchoalveolar lavage or bronchial biopsy (548). Methacholine airway responsiveness in subjects with asthma was similar in subjects with and without eosinophil presence in the airway (189). My study found that both the frequency and severity of AHR in children with eosinophilic asthma was higher compared to children with PGA, suggesting that eosinophils play an important role in AHR in childhood asthma. This study used hypertonic saline, which is an indirect acting stimulus that may be more closely related to inflammatory processes than direct acting stimuli such as methacholine.

7- The ability of tests to detect eosinophilic pattern in children with asthma

Eosinophilic airway inflammation is correlated with the severity of asthma and a favorable response to corticosteroid treatment. Induced sputum can categorize the pattern of airway inflammation but the success rate is less than other non-invasive methods and access to the technique is more difficult. I questioned whether other clinical or objective
features of asthma may predict the EA phenotype. Exhaled nitric oxide is a marker of
eosinophil activation in the airways, but it is also a marker of atopy, which may limit its
specificity for EA. This study found that an elevated FeNO (>25 ppb) had a high
sensitivity (84.6%) but a low specificity (68.8%) for eosinophilic asthma. However, the
combination of high FeNO and other characteristics of asthma such as atopy, a high
asthma control score, airway obstruction, and AHR raised both the sensitivity and
specificity of FeNO to detect an eosinophilic pattern in children with asthma. The highest
sensitivity and specificity were achieved by adding FeNO (>25 ppb) to FEV₁/ FVC ratio
(<80%). In sum, while single clinical asthma features were not good prediction of EA, a
high FeNO combined with the presence of one of asthma characteristic improves the
specificity of test to predict increased sputum eosinophils in children with asthma.
However, the sample size of this study was small, and further study with a greater
number of children is needed to validate these results.

8- Cytokine mediators
IL-8 and TNF- α are known as cytokines which have potentially neutrophil
chemoattractants (162, 500), which explains the significant correlation between sputum
neutrophils and IL-8 and TNF- α. However, there were no different in both % sputum
neutrophils and absolute sputum neutrophils between children with EA and children with
PGA, which resulted in no difference in sputum IL-8 levels between two phenotypes of
airway inflammation in children with asthma. This provides further support for the
absence of neutrophilic mediated processes in stable childhood asthma.
**Conclusion**

Asthma is accepted as a complex disease which many cells take part. There is heterogeneity of airway inflammation in childhood asthma where eosinophilic asthma and paucigranulocytic asthma are the common phenotypes in children. They have different clinical features which may facilitate detection of the phenotypes in clinical practice.

The clinical characteristics of children with PGA were less severe compared to children with EA, as a result of no or little change in airway inflammation in children with PGA compared to healthy children. On the other hand, in subjects with eosinophilic asthma, increased eosinophils related to increased asthma severity. Macrophages may have a role in the inhibition of airway inflammatory processes in children with asthma.

Eosinophilic asthma in children can be detected by the increased FeNO levels in combination with the presence of asthma characteristics such as atopy, low lung function, and AHR. The observations may prove useful for future studies of mechanisms and asthma therapy.
CHAPTER III

INFLUENCE OF PASSIVE SMOKING ON AIRWAY INFLAMMATION IN CHILDREN WITH ASTHMA

Introduction

The harmful effect of tobacco smoke on human health has been well reported. According to the World Health Organization, there were approximately 1.25 billion smokers all over the world, with two-thirds living in developing countries (252). A survey in Australia in 2002 found that 20% of women reported smoking during pregnancy (253).

Environmental tobacco smoke (ETS) exposure is perhaps the most ubiquitous and hazardous to children's health. Secondhand smoke contains the same toxic substances as identified in mainstream tobacco smoke, and children whose parents smoke have been estimated to receive a nicotine dose which is equivalent to actively smoking between 60 and 150 cigarettes per year (549). The influence of passive smoking on the health of children had been mentioned in many studies, especially the relationships between passive smoking and childhood disorders such as prenatal damage to the fetus, poor growth indicators, respiratory illness, middle ear disease, asthma, and sudden infant death syndrome (250, 549).

Epidemiological studies have found that the prevalence of childhood asthma is higher among children who live with smoking parents, more so when both parents were smokers in comparison with those living with non-smoking parents (257). Passive smoking was commonly associated with increased asthma symptoms (133, 550), increased frequency
of asthma exacerbations (250, 337) and increased hospital visits due to asthma (250). Children with asthma living with smoking parents also failed to receive adequate asthma management (266).

Active smoking modifies the pathophysiological features of asthma. Previous studies have described an increased severity of asthma symptoms (305, 311), alterations of airway inflammation (348), and insensitivity to corticosteroids (551) in active smokers with asthma. Elevated sputum neutrophils and/or reduced sputum eosinophils have been observed in asthmatic smokers (348). However, the effects of passive smoking on airway inflammation in asthma are not known, especially in children with asthma.

**Aims**

The aims of this study were to investigate the affects of passive smoking on children with asthma, including asthma symptoms and airway inflammation.

**Hypothesis**

We hypothesized that children with asthma who have ETS exposure may have more severe asthma like active smokers with asthma, including increased asthma symptoms, and a non-eosinophilic pattern of airway inflammation.
Methods

Study design

The study used both cross-sectional and longitudinal designs. Children with asthma were invited to attend between one and three visits. The interval between visits was 3 months. At initial visit, the parents of children with asthma and their child were asked a series of questions about the children’s asthma symptoms and children’s passive smoking exposure. Asthma control was assessed using the Juniper asthma control questionnaire at each visit (403).

Children were required to perform exhaled nitric oxide measurements, exhaled carbon monoxide, exhaled breath condensate, spirometry, hypertonic saline challenge and sputum induction. Skin prick tests were performed at visit 1. Urine samples were collected from the children at each visit in order to assess environmental tobacco smoke exposure.

Subjects

Children with asthma were recruited from the Outpatient clinics at John Hunter Children’s Hospital (Newcastle, NSW, Australia) and by advertisement. All children were aged between 7-17 years. Children with stable asthma were previously diagnosed by paediatricians based on clinical and lung function criteria, no increase in asthmatic symptoms or asthma medication use, no treatment with oral corticosteroid, no unscheduled visits to a GP or hospital due to asthma worsening in the preceding 4 weeks. Children studied in this Chapter represented the same cohort as outlined in Chapter I and II.
**Measurements**

*Environmental Tobacco Smoke Exposure*

Parents and their children were asked a series of questions on smoking. These included: who smoked, the number of cigarettes smoked per day, where they smoked: inside or outside, estimated ETS exposure on children. The questionnaires were modified from previous publications (404, 410).

Measurement of exhaled CO was performed at each visit using the piCO Smokerlyzer® (Bedfont Scientific Ltd, Kent, ME1 3QX England) expressed in parts per million (ppm). Subjects were required to inhale and hold their breath for 15 seconds, then to exhale slowly and gently into the mouthpiece. Exhalation continued to residual volume. The highest level of exhaled CO was recorded (552).

We also collected children’s urine to measure urinary cotinine using Nic-Alert test strips (Nymox Pharmaceutical Corporation, Maywood, NJ). A semi-quantitative scale was used where a Nic-Alert level 0 (0-10 ng/ml) corresponds to no exposure to ETS. A reading of level 1 (10-30ng/ml) is defined as low passive nicotine exposure. A reading of level 2 (30-100ng/ml) indicates higher passive exposure. Level 3 (100-200ng/ml) is the cut-off for assessing if the subject is a smoker. ETS exposure was defined as urinary cotinine in range 10- 100 ng/ml (437).
**Nitric Oxide Measurements**

Measurements of fractional exhaled nitric oxide were made according to ATS guidelines (447). The technique has been described in Methods chapter.

**Skin prick test**

Allergen sensitization to four common allergens, Dermatophagoides pteronyssinus (DP), Alternaria tenuis, cockroach mix and mixed grass was determined by skin prick testing. A positive reaction was defined as a weal diameter equal or larger than 3 mm. Atopy was determined as the presence of at least one positive skin reaction.

**Measurement of lung function**

Spirometry was performed using a spirometer according to ATS guidelines (424). The predicted lung function values of Hibbert et al were used as reference values (425).

**Exhaled breath condensate**

Children were asked to breathe normally with tidal breathing for 10 minutes into a mouthpiece attached to a cooling system. As the children exhaled, the air was cooled and condensed (441). EBC samples were collected and then EBC pH was measured immediately, using a silicon chip sensor.

**Saline challenge and sputum induction**

After spirometry, combined saline challenge and sputum induction were performed (178). Children were asked to produce a specimen of sputum in the sterile container. The
procedure of combined saline challenge and sputum induction has been described in Methods chapter.

_Sputum processing_

Sputum was selected from saliva and processed as described (178, 426). A differential cell count was obtained from 400 cells counted in a cytospin preparation. Eosinophils were expressed from slides stained with Chromotrope 2R in the same fashion.

_IL-8 measurement_

IL-8 in the sputum supernatant was assayed by a sandwich ELISA technique using the Human DuoSet ELISA Kit (IL-8 ELISA; R & D Systems, Inc. Minneapolis MN, USA) with a standard curve range from 31.2pg/mL to 2000pg/mL.

_TNF-α measurement_

Human Tumor Necrosis Factor alpha (TNF-α) was assayed by the sandwich ELISA technique using the DuoSet ELISA Development Kit for TNF-α Cat no: DY 210 (R & D Systems, Inc. Minneapolis MN, USA) with a standard curve range from 31.3pg/mL to 1000pg/mL.

_Statistical methods_

Statistical analysis was carried out using STATA (STATA Corporation, College Station, Texas, USA). Characteristics of the study population and parametric results were expressed as geometric mean and standard deviation (SD). Non parametric results were
reported as median and interquartile range (IQR). For variables with normal distribution, Students t-test was performed. An unpaired t-test was used to analyze data from different subjects whereas a paired t-test was used to compare variables in the same subjects. Nonparametric data was analyzed by the Wilcoxon test for the two groups and by the Kruskal-Wallis test for more than two groups or log transformation for normal distribution. Chi- squared test and Fischer’s exact test was used for categorical data. Case and control groups were compared to estimate odds ratio and the 95% confidence intervals (95%CI). A p < 0.05 was accepted as statistically significant.
Results

**CROSS SECTIONAL STUDIES**

*Parental smoking and ETS exposure in children with asthma*

Sixty six children with asthma and their parents participated in this study. Twenty parents (30.3%) reported tobacco smoking at home. We divided children with asthma into 2 groups based upon parental reported smoking.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parental report</strong></td>
<td></td>
</tr>
<tr>
<td>Either father or mother smoked</td>
<td>30.3%</td>
</tr>
<tr>
<td>Father smoked only</td>
<td>13.6 %</td>
</tr>
<tr>
<td>Mother smoked only</td>
<td>7.6 %</td>
</tr>
<tr>
<td>Both father and mother smoked</td>
<td>9.1 %</td>
</tr>
<tr>
<td>Number of cigarettes smoked daily, median (range)</td>
<td>15 (3-30)</td>
</tr>
</tbody>
</table>

Table 3.3.1 shows that there was a high prevalence of smoking in parents of children with asthma. One parent was a smoker in 21.2% of cases, and both parents smoked in 9.1%. In the group of parental smokers, 45% reported that only the father smoked, whereas in 25% the mother alone was a smoker, and 30% of smoking parents indicated that both the father and mother smoked. The median (IQR) number of cigarettes smoked was 15 (3-30) daily.

Exhaled CO represents recent and significant tobacco smoke exposure, and can be used to detect active smokers. In children with asthma, the median exhaled CO level was 1
ppm and 98.8% of the children with asthma had exhaled CO levels less than 5 ppm. The median exhaled CO level (IQR) of children living with non smoking parents was 1 ppm (1-1), and exhaled CO was 1ppb (1-3) in childhood asthma living with parental smokers. There was no difference in exhaled CO levels between the two groups (p=0.4). The results indicate no active, recent smokers among the children.

Table 3.3. 2: ETS exposure in children with asthma by urinary cotinine

<table>
<thead>
<tr>
<th>Nic- alert level</th>
<th>Concentration (ng/ml)</th>
<th>Percentage ETS exposure (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0-10</td>
<td>53.7 (22)</td>
</tr>
<tr>
<td>1</td>
<td>10-30</td>
<td>31.7 (13)</td>
</tr>
<tr>
<td>2</td>
<td>30-100</td>
<td>14.6 (6)</td>
</tr>
<tr>
<td>3</td>
<td>100-200</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

ETS exposure was established based on urinary cotinine levels at the initial visit. Forty one urine samples were collected. Approximately 54% of children were not exposed to ETS, whereas ETS exposure was detected in 46% of children with asthma. In the ETS exposed group, 31.7% demonstrated low level ETS exposure and 14.6% had high level ETS exposure. No children were a smoker (urinary cotinine ≥ 3).

Relationship between parental smoking and urinary cotinine in children

Sixty seven percent of children living with smoking parents had a positive urinary cotinine result compared to 34.6% of children living with non smoking parents. The prevalence of ETS exposure was significantly higher in children with asthma living with
a smoking parent compared to those without parental smoking (66.7% versus 34.6%; p=0.048).

Table 3.3: Relationship between parents reported smoking and ETS exposure in children with asthma

<table>
<thead>
<tr>
<th>Smoking data</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Either father or mother smoking</td>
<td>3.8</td>
<td>1.6-9.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Father smoking</td>
<td>3.1</td>
<td>1.15-8.4</td>
<td>0.012</td>
</tr>
<tr>
<td>Mother smoking</td>
<td>3.1</td>
<td>1.0-10.2</td>
<td>0.027</td>
</tr>
<tr>
<td>Both father and mother smoking</td>
<td>6.0</td>
<td>1.4-35.9</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table 3.3 describes the relationship between reported parental smoking and ETS exposure in their children. Children living with smoking parents had a significantly increased risk of ETS exposure compared to children living with non-smoking parents (OR=3.8; 95% CI: 1.6-9.2; p=0.008). In addition, if both the father and mother smoked, the odds for ETS exposure in their children was increased to 6 fold (OR=6.0; 95%CI: 1.4-35.9; p=0.005). Either mothers or fathers who smoked can affect on ETS exposure in their children (OR =3.1, 95% CI: 1.0-10.02, p=0.027; OR= 3.1, 95% CI: 1.15-8.4, p=0.012; respectively).

Table 3.4: Correlation between smoking location and ETS exposure in children with asthma

<table>
<thead>
<tr>
<th>Parents report</th>
<th>ETS exposure (-)</th>
<th>ETS exposure (+)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking inside</td>
<td>20 %</td>
<td>80 %</td>
<td>0.08 $^\dagger$</td>
</tr>
<tr>
<td>Smoking outside</td>
<td>25 %</td>
<td>75 %</td>
<td>0.01 $^#$</td>
</tr>
<tr>
<td>No smoking</td>
<td>63 %</td>
<td>37 %</td>
<td>0.005 $^#$</td>
</tr>
</tbody>
</table>

$^# = $ Pearson Chi$^2$ Test
$^\dagger = $ Fisher’s Exact Test
When parents reported that they smoked or permitted smoking inside their houses, the prevalence of ETS exposure in children with asthma was very high (80%; Table 3.3.4). ETS exposure tended to be higher in children whose parents smoked inside the house compared to children living with non-smokers (p=0.08). However, the number of parents who reportedly smoked inside the house was small (n=5). When smoking was permitted outside the house, 75% of children with asthma were found to be exposed to ETS. The prevalence of ETS exposure in children with asthma whose parents only smoked outside was also significantly higher than children living with non-smoking parents (75% versus 37%; p=0.01). When parents were non-smokers, 37% of children with asthma had positive urinary cotinine. Children living with non-smoking parents were less frequently exposed to ETS compared to children living with smokers (p=0.005).

**Parental smoking and clinical asthma features**

**Table 3.3.5: Demographic characteristic of children with asthma with and without parental smoking**

<table>
<thead>
<tr>
<th>Subject data</th>
<th>Children living with non smoking parents (n=46)</th>
<th>Children living with parental smoking (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean, SD; years)</td>
<td>11±3</td>
<td>11±3.6</td>
<td>0.93*</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>47.8</td>
<td>70</td>
<td>0.1#</td>
</tr>
<tr>
<td>Height (mean, SD; cm)</td>
<td>148±14.7</td>
<td>147.4±18.4</td>
<td>0.82*</td>
</tr>
<tr>
<td>Weight (mean, SD, kg)</td>
<td>46.6±17.7</td>
<td>46.2±19.7</td>
<td>0.8*</td>
</tr>
<tr>
<td>BMI (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24.9</td>
<td>78.3</td>
<td>80</td>
<td>0.58$</td>
</tr>
<tr>
<td>≥ 25</td>
<td>21.7</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

* = Unpaired t test
# = Pearson Chi² Test
$ = Fisher’s Exact Test
The demographic characteristics of children with asthma are described in Table 3.3. 5. There was no differences in age, gender, height, weight and BMI between children with asthma living with and without parental smoking.

Table 3.3.6: Clinical characteristics of children with asthma living with and without parental smoking

<table>
<thead>
<tr>
<th>Clinical characteristics over past 12 months (% Subjects)</th>
<th>Childhood asthma living with non smoking parents (n=46)</th>
<th>Childhood asthma living with smoking parents (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Asthma Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>4.3</td>
<td>0</td>
<td>0.041 $</td>
</tr>
<tr>
<td>1-2 year</td>
<td>56.5</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>2-3 year</td>
<td>8.7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>3-4 year</td>
<td>2.2</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>&gt; 4 years</td>
<td>28.3</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Wheezing</td>
<td>78.3</td>
<td>80</td>
<td>0.58 $</td>
</tr>
<tr>
<td>Breathlessness</td>
<td>69.6</td>
<td>80</td>
<td>0.29 $</td>
</tr>
<tr>
<td>Dry cough at night</td>
<td>78.3</td>
<td>85</td>
<td>0.39 $</td>
</tr>
<tr>
<td>Waken by asthma symptoms</td>
<td>34.8</td>
<td>45</td>
<td>0.3 #</td>
</tr>
<tr>
<td>Wheezing during exercise</td>
<td>60.9</td>
<td>85</td>
<td>0.046 $</td>
</tr>
<tr>
<td>Breathlessness at night</td>
<td>45.7</td>
<td>30</td>
<td>0.24 #</td>
</tr>
<tr>
<td>Chest cold</td>
<td>89.1</td>
<td>85</td>
<td>0.46 $</td>
</tr>
<tr>
<td>Asthma attacks</td>
<td>58.7</td>
<td>65</td>
<td>0.63 #</td>
</tr>
<tr>
<td>Visit GP due to asthma attacks</td>
<td>39.1</td>
<td>35</td>
<td>0.75 #</td>
</tr>
<tr>
<td>Absences from school due to asthma</td>
<td>63</td>
<td>60</td>
<td>0.8 #</td>
</tr>
</tbody>
</table>

# = Pearson Chi$^2$ Test
$=$ Fisher’s Exact Test
Asthma was diagnosed at earlier age in those children whose parents were non smokers (p=0.041). More than 60% of childhood asthma was diagnosed before 2 years of age in the group without parental smoking compared to 35% of children with asthma with parental smoking. Conversely, about 30% of children with asthma living with non smoking parent were diagnosed asthma after 3 years of age compared with 55% of children living with a smoking parent.

Asthma symptoms in children with asthma in the last 12 months are described in table 3.3.6. There were no differences in asthma symptoms such as the presence of wheeze, breathlessness, dry cough at night, night waking due to asthma symptoms, breathlessness at night, asthma attacks, GP visits due to asthma attacks, absences from school due to asthma, chest cold in the last 12 months between children with asthma who were living with or without a smoking parent (all p>0.05). Children with asthma living with a smoking parent reported a significantly higher frequency of wheeze during exercise in comparison with those living with non smoking parents (85% versus 60.9%, p=0.046).
Table 3.3.7: History of children with asthma

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children with asthma living with non smoking parents (n=46)</th>
<th>Children with asthma living with smoking parents (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Personal history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eczema</td>
<td>40</td>
<td>20</td>
<td>0.14 $</td>
</tr>
<tr>
<td>Gastro-oesophageal reflux</td>
<td>10.9</td>
<td>15</td>
<td>0.46 $</td>
</tr>
<tr>
<td>Allergic Rhinitis</td>
<td>52.2</td>
<td>55</td>
<td>0.83 #</td>
</tr>
<tr>
<td>Allergies</td>
<td>52.2</td>
<td>50</td>
<td>0.87 #</td>
</tr>
<tr>
<td><strong>Maternal history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>40</td>
<td>50</td>
<td>0.32 #</td>
</tr>
<tr>
<td>Hay fever</td>
<td>52.2</td>
<td>45</td>
<td>0.59 #</td>
</tr>
<tr>
<td>Eczema</td>
<td>19.6</td>
<td>35</td>
<td>0.18 #</td>
</tr>
<tr>
<td>Allergy</td>
<td>47.8</td>
<td>40</td>
<td>0.56 #</td>
</tr>
<tr>
<td>Chest diseases</td>
<td>10.9</td>
<td>10</td>
<td>0.64 $</td>
</tr>
<tr>
<td><strong>Sibling’s diseases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>47.8</td>
<td>45</td>
<td>0.83 #</td>
</tr>
<tr>
<td>Eczema</td>
<td>26.1</td>
<td>5</td>
<td>0.043 $</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>30.4</td>
<td>15</td>
<td>0.16 $</td>
</tr>
<tr>
<td><strong>Living in the same house with smokers when the child was a baby</strong></td>
<td>10.9</td>
<td>90</td>
<td>0.0001 $</td>
</tr>
</tbody>
</table>

# = Pearson Chi$^2$ Test
$ = Fisher’s Exact Test

The history of children with asthma living with and without parental smoking and their family are described in table 3.3.7. A personal history of eczema, allergic rhinitis, and allergies was similar between the two smoking exposed groups (p>0.05). Similarly, there were no differences in maternal history of allergic diseases reported. However, the
prevalence of eczema in siblings of children living without a smoking parent was significantly higher than those living with parental smoking (26.1% versus 5%, p=0.043), despite the prevalence of asthma (47.8% versus 45%, p=0.83) and allergic rhinitis (30.4% versus 15%, p=0.16) of siblings being similar between two groups. The frequency of children with asthma living in the same house with smokers when the child was a baby was significantly higher in the group having parental smoking (90% versus 10.9%, p=0.0001).

Table 3.3.8: Asthma triggers

<table>
<thead>
<tr>
<th>Characteristics (%) Subjects</th>
<th>Children with asthma living with non smoking parents (n=46)</th>
<th>Children with asthma living with smoking parents (n=20)</th>
<th>P value #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral infection</td>
<td>93.5</td>
<td>55</td>
<td>0.001</td>
</tr>
<tr>
<td>Allergy exposure</td>
<td>60.9</td>
<td>40</td>
<td>0.12</td>
</tr>
<tr>
<td>Exercise induced</td>
<td>69.6</td>
<td>70</td>
<td>0.97</td>
</tr>
<tr>
<td>Cigarette smoke</td>
<td>32.6</td>
<td>45</td>
<td>0.4</td>
</tr>
<tr>
<td>Atmospheric pollutants</td>
<td>50</td>
<td>30</td>
<td>0.13</td>
</tr>
<tr>
<td>Weather change</td>
<td>67.4</td>
<td>80</td>
<td>0.23</td>
</tr>
<tr>
<td>Emotional stress</td>
<td>43.5</td>
<td>40</td>
<td>0.79</td>
</tr>
</tbody>
</table>

# = Pearson Chi² Test
The occurrence of asthma symptoms in response to triggers is reported in table 3.3.8 and figure 3.3.1. The results indicate that children living with non-smoking parents were more likely to have their asthma triggered by viral infection than those living with smoking parents (93.5% versus 55%, p=0.001). Allergy exposure tended to trigger asthma in subjects living without smokers rather than in subjects living with smokers (60.9% versus 40%, p=0.12) but this result was not statistically significant. Other asthma triggers such as exercise, cigarette smoke, atmospheric pollutants, weather change, and emotional stress were similar in childhood asthma who lived with and without smoking parents (p>0.05).
<table>
<thead>
<tr>
<th>Characteristics (median, IQR)</th>
<th>Children with asthma living with non smoking parents (n=44)</th>
<th>Children with asthma living with smoking parents (n=19)</th>
<th>P value $^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woken by asthma</td>
<td>0 (0-0)</td>
<td>0 (0-0.5)</td>
<td>0.37</td>
</tr>
<tr>
<td>Symptom upon waking</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
<td>0.68</td>
</tr>
<tr>
<td>Activities limited by asthma</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0.96</td>
</tr>
<tr>
<td>SOB due to asthma</td>
<td>0(0-1)</td>
<td>1 (0-2)</td>
<td>0.35</td>
</tr>
<tr>
<td>Wheeze</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
<td>0.65</td>
</tr>
<tr>
<td>SABA puffs per day</td>
<td>0 (0-1)</td>
<td>1(1-2)</td>
<td>0.16</td>
</tr>
<tr>
<td>FEV$_{1}$% predicted</td>
<td>1(0-2)</td>
<td>1(0-2)</td>
<td>0.63</td>
</tr>
<tr>
<td>Overall score</td>
<td>0.42 (0.14-1)</td>
<td>0.57 (0.14-1)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

$^+$ = Mann-Whitney Test  
SOB = shortness of breath  
SABA: short acting $\beta_2$ agonist

Asthma control score was assessed as described above (Table 3.3.9), using the Juniper asthma control questionnaire (403). Sixty three children with asthma (95.5%) completed this questionnaire. Both groups had well controlled asthma, with median (IQR) asthma control scores of 0.43 (0.14-1) for children living with non smokers and 0.57 (0.14-1) for children living with smokers. The overall asthma control score was lower in children living with non smoking parents compared with those living with smoking parents, but it failed to reach statistical significance (0.43 versus 0.57, p=0.65). When a comparison of the individual items was performed, the data showed similar scores for asthma symptoms such as woken up by asthma at night, asthma symptoms when waking up, activities
limited by asthma, and wheeze between the two groups. The group living with smoking parents was likely to have more breathlessness due to asthma and to require more short acting $\beta_2$ agonists than the group without parental smoking, but again these results did not reach to statistical significance ($p=0.35$, $p=0.16$; respectively).

Table 3.3.10: Clinical pattern of children with asthma with and without parental smoking

<table>
<thead>
<tr>
<th>Characteristics (% Subjects)</th>
<th>Children with asthma living with non smoking parents (n=46)</th>
<th>Children with asthma living with smoking parents (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infrequent episodic</td>
<td>34.8</td>
<td>20</td>
<td>0.18 $^S$</td>
</tr>
<tr>
<td>Frequent episodic</td>
<td>39.1</td>
<td>40</td>
<td>0.95 $^#$</td>
</tr>
<tr>
<td>Persistent</td>
<td>26.1</td>
<td>40</td>
<td>0.26 $^#$</td>
</tr>
</tbody>
</table>

Overall score: 0.37 (Fisher’s Exact Test)

$^#$ = Pearson Chi$^2$ Test

$^S$ = Fisher’s Exact Test

Figure 3.3.2: Clinical pattern of childhood asthma with and without parental smoking
The clinical pattern of children with asthma was categorized using the National Asthma Council Australia guidelines (553). Table 3.3.10 and figure 3.3.2 report the clinical pattern of asthma in children living with and without smoking parents. The percentages of infrequent episodic, frequent episodic and persistent asthma in subjects living with and without smokers were 20% versus 34.8%; 40% versus 39.1%, and 40% versus 26.1%, respectively. Children with asthma living with parental smoking seemed to have more persistent asthma compared to those living with non smoking parents, but they failed to reach statistical significance ( p= 0.26).

*Figure 3.3. 3: Parental smoking in childhood asthma, by clinical asthma pattern*

Parental smoking was more common in children with more severe asthma. Twenty percent of children were reported living with smokers in the infrequent group, it raised to 31% in the frequent group, and 40% in the persistent asthma, p=0.15.
Asthma treatment is shown in the table 3.3.11. There was no difference in ICS use between children with asthma living with and without parental smoking (65% versus 63%, $p=0.88$). The median ICS dose (IQR) for maintenance asthma therapy for subjects living with smoking parents was $300 \mu g/day$ (0-1000) in comparison with $225 \mu g/day$ (0-500) for subjects living with non smoking parents ($p=0.48$). The children in the group with parental smoking were likely to use Leukotriene antagonists (Montelukast) compared to children living without parental smoking (15% versus 2.2%, $p=0.08$), but the number of subjects was very small (n=4).
Exhaled carbon monoxide

Table 3.3.12: Exhaled carbon monoxide in children with asthma living with and without parental smoking

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children with asthma living with non smoking parents (n=45)</th>
<th>Children with asthma living with smoking parents (n=20)</th>
<th>P value $^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhaled carbon monoxide ppm; median (IQR)</td>
<td>1 (1-1)</td>
<td>1 (1-2.5)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

$^+$ = Mann-Whitney Test

Measurement of exhaled carbon monoxide was performed successfully in 45 (98%) children with asthma without parent smoking and 20 (100%) children with asthma with parent smoking. There was no difference in exhaled carbon monoxide levels between children with asthma living with and without parental smoking (1 ppm versus 1 ppm, p=0.77; Table 3.3.12).

Atopy

Table 3.3.13: Atopic status in children with asthma with and without parental smoking

<table>
<thead>
<tr>
<th>Characteristics (%) subjects</th>
<th>Children with asthma living with non smoking parents (n=46)</th>
<th>Children with asthma living with smoking parents (n=19)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopy</td>
<td>80.4</td>
<td>80</td>
<td>0.57 $^*$</td>
</tr>
<tr>
<td>Alternaria</td>
<td>37</td>
<td>21</td>
<td>0.17 $^*$</td>
</tr>
<tr>
<td>Dust mite</td>
<td>78.3</td>
<td>73.7</td>
<td>0.69 $^*$</td>
</tr>
<tr>
<td>Cockroach</td>
<td>40</td>
<td>15.8</td>
<td>0.08 $^*$</td>
</tr>
<tr>
<td>Grass mix</td>
<td>63</td>
<td>31.2</td>
<td>0.02 $^*$</td>
</tr>
</tbody>
</table>

$^*$ = Pearson Chi$^2$ Test
$^*$ = Fisher’s Exact Test
Atopic status in children with asthma is described in table 3.3.13. The overall prevalence of atopy was similar between two groups (80.4% versus 80%, p=0.57). No differences in sensitization to Alternaria, Dust mite, and Cockroach were found between children with asthma with and without parental smoking. Significantly more children living with non-smoking parents were sensitized to Grass mix than children living with smoking parents (63% versus 31.2%, p=0.02). The prevalence of sensitization to more than one allergen was also a significantly higher in children living with non-smoking parents compared to children living with smoking parents (91.4% versus 60%, p=0.015).

*Fractional exhaled Nitric oxide*

*Figure 3.3.4: Fractional exhaled Nitric Oxide in children with asthma living with and without parental smoking*

Fractional exhaled Nitric Oxide was measured in 17 (85%) subjects living with smoking parents and 34 (74%) subjects living with non-smoking parents. No difference in FeNO levels were found between the two groups (27.5 ppb versus 23.65 ppb, p=0.72).
Lung function

Table 3.3.14: Lung function of children with asthma with and without parental smoking

<table>
<thead>
<tr>
<th>Characteristics ( % subjects)</th>
<th>Children with asthma living with non smoking parents (n=44)</th>
<th>Children with asthma living with smoking parents (n=19)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;% predicted mean (SD)</td>
<td>91.7±13.1</td>
<td>92.4±13.4</td>
<td>0.72 *</td>
</tr>
<tr>
<td>% FEV&lt;sub&gt;1&lt;/sub&gt;/FVC mean (SD)</td>
<td>81.3±9.3</td>
<td>82.3±7</td>
<td>0.58 *</td>
</tr>
</tbody>
</table>

* = Unpaired t test

Ninety five percent of children with asthma having smoking parents and 96% of children with asthma without smoking parents were able to perform spirometry. The mean (SD) FEV<sub>1</sub>% predicted for children without smoking parents was 91.7% (13.1) compared with 92.4% (13.4) for children with smoking parents (p=0.72). The FEV<sub>1</sub>/FVC ratio was similar between the two groups (81.3% versus 82.3%, p=0.58).

Airway hyperresponsiveness

Table 3.3.15: AHR in childhood asthma with and without smoking parents

<table>
<thead>
<tr>
<th>Characteristics ( % subjects)</th>
<th>Children with asthma living with non smoking parents (n=43)</th>
<th>Children with asthma living with smoking parents (n=19)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHR (%)</td>
<td>62.8</td>
<td>52.6</td>
<td>0.45 #</td>
</tr>
<tr>
<td>PD&lt;sub&gt;15&lt;/sub&gt;, mL median (IQR)</td>
<td>2.8 (1.3-6.82)</td>
<td>2.83 (0.72-6.26)</td>
<td>0.9 +</td>
</tr>
</tbody>
</table>

# = Pearson Chi<sup>2</sup> Test
+ = Mann-Whitney Test

AHR was assessed using hypertonic saline (4.5%) challenge. Saline challenge was performed in 43 (93.5%) children with asthma living with non smoking parents and 19
(95%) children with asthma living with a smoking parent. The prevalence of AHR for children with asthma living with smoking parents was 52.6% compared to 62.8% for the children living with non smoking parents (p=0.45). The PD_{15} was similar in the two groups (2.8 ml versus 2.83 ml, p=0.9).

*Exhaled breath condensate*

*Figure 3.3.5: pH of EBC in children with asthma with and without parental smoking*

![Figure 3.3.5: pH of EBC in children with asthma with and without parental smoking](image)

EBC samples were collected in 19 (95%) children living with parental smokers and 46 (100%) children living with non smoking parents. The median EBC pH (IQR) was 7.2 (6.8-7.8) for subjects living with parental smoking and 7.45 (7.1-7.8) for subjects living with non parental smoking, but it failed to reach statistical significance (p=0.56).
Table 3.3.16: Induced sputum cell counts in children with asthma with and without parental smoking

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children with asthma living with non smoking parents (n=28)</th>
<th>Children with asthma living with smoking parents (n=14)</th>
<th>P value +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality, median (IQR)</td>
<td>17 (13-19)</td>
<td>16.5 (15-18)</td>
<td>0.92</td>
</tr>
<tr>
<td>Viability %, median (IQR)</td>
<td>67.31 (42.8-88.1)</td>
<td>71.5 (60.4-82.3)</td>
<td>0.41</td>
</tr>
<tr>
<td>TCC x 10⁹/ml, median (IQR)</td>
<td>2.25 (1.04-6.66)</td>
<td>2.84 (1.49-5.27)</td>
<td>0.67</td>
</tr>
<tr>
<td>Neutrophils %, median (IQR)</td>
<td>13.38 (6.25-40.38)</td>
<td>11.75 (4.75-28.25)</td>
<td>0.81</td>
</tr>
<tr>
<td>Absolute neutrophils x 10⁹/ml median (IQR)</td>
<td>26.25 (7.55-259.58)</td>
<td>33.05 (6.75-314.69)</td>
<td>0.74</td>
</tr>
<tr>
<td>Eosinophils %, median (IQR)</td>
<td>3.25 (0.25-16.25)</td>
<td>2.5 (0-7.85)</td>
<td>0.36</td>
</tr>
<tr>
<td>Absolute eosinophils x 10⁹/ml median (IQR)</td>
<td>7.56 (2.84-35.10)</td>
<td>5.67 (0-16.08)</td>
<td>0.43</td>
</tr>
<tr>
<td>Macrophage % median (IQR)</td>
<td>67.10 (39-80.85)</td>
<td>68.0 (55.56-90.57)</td>
<td>0.56</td>
</tr>
<tr>
<td>Absolute macrophage x 10⁹/ml median (IQR)</td>
<td>127.92</td>
<td>164.25</td>
<td>0.74</td>
</tr>
<tr>
<td>Lymphocyte %, median (IQR)</td>
<td>1.0 (0.5-1.88)</td>
<td>0.5 (0.25-1.75)</td>
<td>0.27</td>
</tr>
<tr>
<td>Epithelial %, median (IQR)</td>
<td>2.5 (0.12-4.90)</td>
<td>1.12 (0.25-2.25)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

+ = Mann-Whitney Test
Sputum samples were obtained in 28 (61%) children with asthma without parental smoking and 14 (70%) children with asthma with parental smoking. Sputum results are described in table 3.3.16. The quality of sputum samples and the viability of sputum cells were the same in the two groups. The median total cell count (IQR) was $2.25 \times 10^6$/ml (1.04-6.66) for subjects without parental smoking compared to $2.84 \times 10^6$/ml (1.49-5.27) for subjects with a parent who was a smoker, $p=0.67$.

Sputum neutrophils were assessed, with no difference in % sputum neutrophils in children with asthma living with and without smoking parents (11.75% versus 13.38%, $p=0.81$). The number of sputum neutrophils were lower in children with asthma with non smoking parents than in subjects with smoking parents, but this failed to reach statistical significance ($26.25 \times 10^4$/ml versus $33.05 \times 10^4$/ml, $p=0.74$).

Although both % sputum eosinophils and number of sputum eosinophils in children with asthma living with non smoking parents were higher than in children with asthma living with a parent smoking, again they were not significantly different (3.25% versus 2.5%, $p=0.36$; $7.56 \times 10^4$/ml versus $5.67 \times 10^4$/ml, $p=0.43$; respectively; Table 3.3.16).

Other sputum cells such as macrophages, lymphocytes, and epithelial cells showed similar percentages between two groups (Table 3.3.16).
### Sputum cytokines

**Table 3.3.17: Comparison in sputum IL-8 between childhood asthma living with and without parental smoking**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children with asthma living with non smoking parents (n=33)</th>
<th>Children with asthma living with smoking parents (n=14)</th>
<th>P value $^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 (ng/ml)</td>
<td>1.67 (0.56-4.17)</td>
<td>2.15 (1.58-5.35)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

$^+$ = Mann-Whitney Test

IL-8 was 2.15 ng/ml for children with asthma living with smoking parents compared to 1.67 ng/ml for those living with non-smoking parents, $p=0.4$ (Table 3.3.17).
Comparison in children with asthma with and without ETS exposure based upon urinary cotinine at the initial visit

Another cross-sectional investigation was performed to compare asthma control and markers of airway inflammation in children with asthma with and without ETS exposure based upon urinary cotinine at the initial visit (Visit 1). Two groups of children were classified: childhood asthma without ETS exposure (negative urinary cotinine) and childhood asthma with ETS exposure (positive urinary cotinine).

Forty-one urine samples were collected at initial visit. Twenty two (53.7%) children with asthma were not exposed to ETS (urinary cotinine: level 0) whereas nineteen (46.3%) children with asthma with ETS exposure (urinary cotinine: level 1 and level 2).

ETS exposure in children with asthma by age

Figure 3.3.6: Percentage of ETS exposed children by age
ETS exposure was investigated based on three age groups (7-9 years; 10-13 years and 14-17 years). The percentage of ETS exposure was 50% for children with asthma in the 7-9 year group, 30.8% for children with asthma in the 10-13 year group and 58.3% for children with asthma aged 14-17 years. The percentage of ETS exposure was similar among the three groups (p=0.39; Figure 3.3.6).

**Asthma control score**

*Table 3.3.18: Asthma control score in children with asthma with and without ETS exposure*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children with asthma without ETS exposure (n=22)</th>
<th>Children with asthma with ETS exposure (n=19)</th>
<th>P value +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woken by asthma</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0.5</td>
</tr>
<tr>
<td>Symptom upon waking</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
<td>0.37</td>
</tr>
<tr>
<td>Activities limited by asthma</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0.87</td>
</tr>
<tr>
<td>SOB due to asthma</td>
<td>1(0-2)</td>
<td>0 (0-2)</td>
<td>0.56</td>
</tr>
<tr>
<td>Wheeze</td>
<td>1(0-1)</td>
<td>0 (0-1)</td>
<td>0.22</td>
</tr>
<tr>
<td>SABA puffs per day</td>
<td>1(0-1)</td>
<td>0(0-1)</td>
<td>0.13</td>
</tr>
<tr>
<td>FEV₁% predicted</td>
<td>1(0-2)</td>
<td>0(0-1)</td>
<td>0.74</td>
</tr>
<tr>
<td>Overall score</td>
<td>0.57 (0.14-1)</td>
<td>0.42 (0.14-1)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

+ = Mann-Whitney Test

The asthma control score was 0.57 for subjects without ETS exposure compared with 0.42 for subjects with ETS exposure (p=0.6). There were no differences for individual
items for assessing asthma control score between children with asthma with and without ETS exposure (Table 3.3.18).

**Exhaled Carbon monoxide**

Table 3.3.19: Comparison of exhaled Carbon monoxide in children with asthma with and without ETS exposure

|                      | Children with asthma without ETS exposure (n=22) | Children with asthma with ETS exposure (n=19) | P value  
|----------------------|-------------------------------------------------|---------------------------------------------|----------
| Exhaled CO, ppm median (IQR) | 1 (1-1)                                         | 1 (1-3)                                    | 0.29     |

+= Mann-Whitney Test

The median exhaled Carbon monoxide (IQR) was 1 ppm (1-1) for childhood asthma without ETS exposure compared to 1 ppm (1-3) for childhood asthma with ETS exposure, p=0.29. Exhaled Carbon monoxide was a significantly higher in subjects with high ETS exposure compared to those without ETS exposure (4 ppm versus 1 ppb, p=0.02). However, the number of children with asthma who were highly exposed to ETS was small (n=6).

**Fractional exhaled Nitric oxide**

Table 3.3.20: Comparison in FeNO levels in children with asthma with and without ETS exposure

|                      | Children without ETS exposure (n=20) | Children with ETS exposure (n=18) | P value  
|----------------------|--------------------------------------|----------------------------------|----------
| FeNO levels, ppb median (IQR) | 25.05 (14.7-42.05)                  | 24.6 (9.8-46.3)                  | 0.67     |

+= Mann-Whitney Test
FeNO measurement was successfully measured in twenty children with asthma without ETS exposure and eighteen children with asthma with ETS exposure. The FeNO level was 25.05 ppb for children with asthma without ETS exposure compared to 24.6 ppb for children with asthma with ETS exposure (p = 0.67; Table 3.3.20, Figure 3.3.7).

Table 3.3.21: FeNO levels in children with asthma with different ETS exposure levels

<table>
<thead>
<tr>
<th></th>
<th>Children without ETS exposure (n=20)</th>
<th>Children with low ETS exposure (n=13)</th>
<th>Children with high ETS exposure (n=5)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeNO level (median, IQR; ppb)</td>
<td>25.05 (14.7-42.05)</td>
<td>27.5 (9.8-46.3)</td>
<td>15 (13.6-45.3)</td>
<td>0.89 Δ</td>
</tr>
</tbody>
</table>

Δ = Kruskal – Wallis Test
When FeNO levels in children with asthma and different ETS exposure levels were evaluated (Table 3.3.21, Figure 3.3.8), the data showed that the FeNO was no different in children with asthma at different ETS exposed levels ($p=0.89$). FeNO levels were decreased in children with asthma who were exposed to high ETS compared to children with asthma without ETS exposure, but this failed to reach a statistical significance (15 ppb versus 25.05 ppb, $p=0.63$). However, the number of children with asthma who had high ETS exposure was small ($n=5$).
**Lung function**

*Table 3.3.22: Lung function in children with asthma with and without ETS exposure*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children without ETS exposure (n=21)</th>
<th>Children with ETS exposure (n=17)</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV$_1$ % predicted %, mean (SD)</td>
<td>93.3 ± 11.9</td>
<td>92.4 ± 15.6</td>
<td>0.85</td>
</tr>
<tr>
<td>FEV$_1$/FVC %, mean (SD)</td>
<td>80.3 ± 8.5</td>
<td>84.8 ± 9</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*= Unpaired t test

Twenty one children with asthma without ETS exposure and seventeen children with asthma with ETS exposure could perform spirometry. FEV$_1$ % predicted (SD) was 92.4% (15.6) for children with asthma with ETS exposure compared to 93.3 % (11.9) for those without ETS exposure, p=0.85. The % FEV$_1$/FVC ratio was also similar between subjects with and without ETS exposure (84.8% versus 80.3%, p=0.12; Table 3.3. 22).

**Airway hyperresponsiveness**

*Table3. 23: AHR in children with asthma with and without ETS exposure*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children without ETS exposure (n=20)</th>
<th>Children with ETS exposure (n=17)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHR (% subjects)</td>
<td>60</td>
<td>53</td>
<td>0.67 #</td>
</tr>
<tr>
<td>PD$_{15}$, ml, median (IQR)</td>
<td>3.77(1.12-6.82)</td>
<td>3.08 (0.72-6.26)</td>
<td>0.69 +</td>
</tr>
</tbody>
</table>

#= Pearson Chi$^2$ Test

+= Mann-Whitney Test
Table 3.3.23 describes AHR in subjects with asthma with and without ETS exposure. Twenty children with asthma without ETS exposure and seventeen children with asthma with ETS exposure performed saline challenge. AHR was present in 60% of children with asthma without ETS exposure and 53% of children with asthma with ETS exposure (p=0.67). The provocation dose causing a 15% fall in FEV$_1$ (PD15) was similar between the two groups (3.77 ml versus 3.08 ml, p=0.69).

**Exhaled breath condensate**

*Table 3.3.24: pH of EBC in children with asthma with and without ETS exposure*

<table>
<thead>
<tr>
<th></th>
<th>Children without ETS exposure (n=20)</th>
<th>Children with ETS exposure (n=19)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH of EBC median (IQR)</td>
<td>7.35 (6.9-7.75)</td>
<td>7.5 (7.2-7.8)</td>
<td>0.54 *</td>
</tr>
</tbody>
</table>

+= Mann Whitney Test

EBC samples were collected in 20 children without ETS exposure and 19 children with ETS exposure. Whether or not they were exposed to ETS, EBC pH was similar between the two group (7.35 versus 7.5, p= 0.54; Table 3.3.24).
### Sputum cell counts

**Table 3.3.25: Induced sputum cell counts in children with asthma with and without ETS exposure**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children without ETS exposure (n=17)</th>
<th>Children with ETS exposure (n=10)</th>
<th>P value $^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality, median (IQR)</td>
<td>18 (16-19)</td>
<td>18 (16-19)</td>
<td>0.87</td>
</tr>
<tr>
<td>Viability % median (IQR)</td>
<td>76.5 (65.83-90.69)</td>
<td>73.49 (66.6-89)</td>
<td>0.9</td>
</tr>
<tr>
<td>TCC x 10⁶/ml median (IQR)</td>
<td>3.06 (1.17-11.71)</td>
<td>1.67 (0.9-3.15)</td>
<td>0.4</td>
</tr>
<tr>
<td>Neutrophils % median (IQR)</td>
<td>22 (6.16-68.25)</td>
<td>17.5 (9.5-26)</td>
<td>0.85</td>
</tr>
<tr>
<td>Absolute neutrophils x10⁴/ml median (IQR)</td>
<td>94.5 (12.24-546.48)</td>
<td>25.515 (7.7-65.5)</td>
<td>0.3</td>
</tr>
<tr>
<td>Eosinophils % median (IQR)</td>
<td>1.34 (0.5-2.6)</td>
<td>4 (3.25-5.25)</td>
<td>0.17</td>
</tr>
<tr>
<td>Eosinophil absolute x 10⁴/ml median (IQR)</td>
<td>5.76 (3.16-15.92)</td>
<td>5.36 (3.24-24.3)</td>
<td>0.8</td>
</tr>
<tr>
<td>Macrophage % median (IQR)</td>
<td>56.75 (20-86.75)</td>
<td>69 (53.5-76)</td>
<td>0.73</td>
</tr>
<tr>
<td>Macrophage absolute x 10⁴/ml median (IQR)</td>
<td>113.93 (66.76-185.63)</td>
<td>116.24 (108.68-123.12)</td>
<td>0.92</td>
</tr>
<tr>
<td>Lymphocyte % median (IQR)</td>
<td>0.5 (0.25-1)</td>
<td>0.25 (0-0.5)</td>
<td>0.25</td>
</tr>
<tr>
<td>Epithelial % median (IQR)</td>
<td>0.75 (0-3)</td>
<td>0.5 (0.25-3)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

$^+ = $ Mann-Whitney Test
Only 17 subjects (77.3%) in the non ETS exposed group and 10 (52.6%) subjects in the ETS exposed group had satisfactory sputum samples to permit an analysis of sputum cell counts (Table 3.3.25).

The quality of sputum and the viability of sputum cells were similar in children with asthma with and without ETS exposure (p=0.87, p=0.9; respectively). TCC was $3.06 \times 10^6$/ml in subjects without ETS exposure compared to $1.67 \times 10^6$/ml in subjects with ETS exposure, p=0.4.

Neither % sputum neutrophils nor absolute sputum neutrophils were different in childhood asthma with and without ETS exposure (17.5% versus 22%, p=0.85; 25.52 $\times 10^4$/ml versus 94.5 $\times 10^4$/ml, p=0.3; respectively). Similarly, % sputum eosinophils and absolute sputum eosinophil were also similar in children with and without exposure to ETS (4% versus 1.34%, p=0.17; 5.36 $\times 10^4$/ml versus 5.76 $\times 10^4$/ml, p=0.8; respectively).

Other sputum cells such as macrophages, lymphocytes, and epithelial cells also demonstrated with similar percentages between children with asthma with and without ETS exposure (p=0.73, 0.25, and 0.99; respectively).
**Sputum cytokines**

*Table 3.3.26: Sputum IL-8 in children with asthma with and without ETS exposure*

<table>
<thead>
<tr>
<th></th>
<th>Children without ETS exposure (n=17)</th>
<th>Children with ETS exposure (n=12)</th>
<th>P value +</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8; ng/ml</td>
<td>2.03 (0.56-4.17)</td>
<td>1.81 (1.02-6.02)</td>
<td>0.36</td>
</tr>
<tr>
<td>median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+= Mann Whitney Test

IL-8 was 2.03 ng/ml for subjects with asthma without ETS exposure compared to 1.81 ng/ml for subjects with ETS exposure, p=0.36.
LONGITUDINAL STUDY

We also investigated the relationship between clinical symptoms and airway inflammatory markers in each child with asthma during different ETS exposed periods (children with asthma without ETS exposure showed a negative urinary cotinine result: urinary cotinine level 0 and children with asthma with ETS exposure demonstrated a positive urinary cotinine result: urinary cotinine level 1 and level 2). Each child with asthma attended between two or three visits. The interval between visits was 3 months. Three groups of children with asthma with different ETS exposed patterns between visits were found. There were twelve children (27.3%) with no ETS exposure at any visit (Non-exposed), twenty six children (59.1%) who had changed their ETS exposed condition from negative to positive between previous and later visits (Varying exposure), and six children (13.6%) who had constant ETS exposure at all visits (Constant exposure).
Table 3.3.27: Clinical symptoms and airway inflammatory markers in children with asthma without ETS exposure at any visit: Non-Exposed

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma control score</td>
<td>0.29 (0.07-0.64)</td>
<td>0.14 (0.07-0.5)</td>
<td>0.58 †</td>
</tr>
<tr>
<td>median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV(_1) % predicted mean (SD)</td>
<td>93.6 ± 11.04</td>
<td>94.18 ± 9.4</td>
<td>0.9 **</td>
</tr>
<tr>
<td>% FEV(_1)/FVC ratio mean (SD)</td>
<td>83.55 ± 6.86</td>
<td>82.91 ± 5.75</td>
<td>0.82 **</td>
</tr>
<tr>
<td>FeNO levels; ppb median (IQR)</td>
<td>35.78 (14.7-54.85)</td>
<td>36.5 (15.1-58.95)</td>
<td>0.81 †</td>
</tr>
<tr>
<td>EBC pH median (IQR)</td>
<td>7.3 (6.7-7.5)</td>
<td>7.1 (6.8-7.5)</td>
<td>0.67 †</td>
</tr>
<tr>
<td>Sputum neutrophils % median (IQR)</td>
<td>9.25 (6.16-41)</td>
<td>7.25 (2.25-64.5)</td>
<td>0.85 †</td>
</tr>
<tr>
<td>Absolute sputum neutrophils x 10(^4)/ml, median (IQR)</td>
<td>36.27 (9.99-2089.8)</td>
<td>88.56 (1.62-307.665)</td>
<td>0.83 †</td>
</tr>
<tr>
<td>Sputum eosinophils; % median (IQR)</td>
<td>2.5 (0.5-3.5)</td>
<td>2.5 (0.25-11)</td>
<td>0.95 †</td>
</tr>
<tr>
<td>Absolute sputum eosinophils x 10(^4)/ml, median (IQR)</td>
<td>6.14 (2.7-16.27)</td>
<td>5.4 (0-52.47)</td>
<td>0.83 †</td>
</tr>
</tbody>
</table>

† = Wilcoxon matched pairs test

** = Paired t test

In 12 children with asthma without ETS exposure at any visit, the asthma symptoms (asthma control score), the objective measurements (lung function) and airway inflammatory markers (FeNO levels, EBC pH, sputum cell counts) were similar between study visits (all p>0.05; Table 3.3.27).
Table 3.3.28: Clinical symptoms and airway inflammatory markers in children with asthma with ETS exposure at all visits: Constant Exposure

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma control score median (IQR)</td>
<td>0.29 (0.14-1)</td>
<td>0.29 (0.14-0.71)</td>
<td>0.98 ‡</td>
</tr>
<tr>
<td>FEV(_1) % predicted mean (SD)</td>
<td>91.6 ± 11.5</td>
<td>91.2 ± 10.9</td>
<td>0.96 **</td>
</tr>
<tr>
<td>% FEV(_1)/FVC ratio mean (SD)</td>
<td>81.6 ± 6.54</td>
<td>79.4 ± 9.9</td>
<td>0.46 **</td>
</tr>
<tr>
<td>FeNO levels, ppb median (IQR)</td>
<td>33.5 (25-48.5)</td>
<td>29 (25.1-57.1)</td>
<td>0.99 ‡</td>
</tr>
<tr>
<td>EBC pH median (IQR)</td>
<td>7.45 (6.8-7.6)</td>
<td>7.35 (6.9-7.6)</td>
<td>0.94 ‡</td>
</tr>
<tr>
<td>Sputum neutrophils, % median (IQR)</td>
<td>5 (4.75-9.5)</td>
<td>5.5 (4.75-32.75)</td>
<td>0.83 ‡</td>
</tr>
<tr>
<td>Absolute sputum neutrophils x 10(^4)/ml, median (IQR)</td>
<td>5.99 (4.05-7.70)</td>
<td>26.23 (2.57-67.79)</td>
<td>0.51 ‡</td>
</tr>
<tr>
<td>Sputum eosinophils, % median (IQR)</td>
<td>2.75 (1.75-35.75)</td>
<td>3.5 (1.75-12.25)</td>
<td>0.99 ‡</td>
</tr>
<tr>
<td>Absolute sputum eosinophils x 10(^4)/ml, median (IQR)</td>
<td>3.47 (1.42-28.96)</td>
<td>8.35 (1.89-25.36)</td>
<td>0.83 ‡</td>
</tr>
</tbody>
</table>

‡ = Wilcoxon matched pairs test

** = Paired t test

There were 6 children with asthma who were exposed to ETS at all study visits. Comparisons in asthma symptoms, lung function and airway inflammatory markers (FeNO levels, EBC pH and sputum cell counts) showed similar results between visits (all p >0.05; Table 3.3.28).
**Effects of alterations in ETS exposure in children with asthma**

Twenty six children with asthma had changed their ETS exposed condition from non exposure to exposure between previous and later visits.

**Asthma control score**

*Table 3.3.29: Asthma control score in children with asthma who had changed ETS exposed condition from non exposure to exposure between previous and later visits.*

<table>
<thead>
<tr>
<th>Characteristics (median, IQR)</th>
<th>Children with asthma without ETS exposure</th>
<th>Children with asthma with ETS exposure</th>
<th>P value ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woken by asthma</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0.63</td>
</tr>
<tr>
<td>Symptom upon waking</td>
<td>0 (0-0)</td>
<td>0 (0-1)</td>
<td>0.16</td>
</tr>
<tr>
<td>Activities limited by asthma</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0.46</td>
</tr>
<tr>
<td>SOB due to asthma</td>
<td>0 (0-1)</td>
<td>0 (0-0)</td>
<td>0.16</td>
</tr>
<tr>
<td>Wheeze</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
<td>0.24</td>
</tr>
<tr>
<td>SABA puffs per day</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
<td>0.25</td>
</tr>
<tr>
<td>FEV₁% predicted</td>
<td>1 (0-1)</td>
<td>2 (0-2)</td>
<td>0.03</td>
</tr>
<tr>
<td>Overall score</td>
<td>0.29</td>
<td>0.42</td>
<td>0.33</td>
</tr>
</tbody>
</table>

‡= Wilcoxon matched pairs test

Asthma control score was completed in 22 children with asthma. The median asthma control score of children with asthma during a non ETS exposed period was 0.29 compared to 0.42 for ETS exposed period, p=0.33, Table 3.3.29.
**Exhaled Carbon monoxide**

Table 3.3.30: Exhaled CO in each child with asthma with and without ETS exposure

<table>
<thead>
<tr>
<th></th>
<th>Children with asthma without ETS exposure</th>
<th>Children with asthma with ETS exposure</th>
<th>P value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhaled CO, ppm median (IQR)</td>
<td>1 (1-1)</td>
<td>2 (1-3)</td>
<td>0.027</td>
</tr>
</tbody>
</table>

‡: Wilcoxon matched pairs test

Twenty five children with asthma had exhaled CO results for both non ETS exposed and ETS exposed periods. Children with asthma during the ETS exposed period had a significantly higher exhaled CO level compared to a non-ETS exposed period (2 ppm versus 1 ppm, p=0.027), but they all were below the level of active smoking.

**Exhaled Nitric oxide**

Figure 3.3. 9: Comparison in FeNO levels in children with asthma with and without ETS exposure.

FeNO levels in the same children at different ETS exposed periods

*p=0.003
Twenty two children with asthma had exhaled NO results at both ETS and non ETS exposed periods. A comparison of FeNO levels in each child with asthma at the different ETS exposed periods found that FeNO levels were significantly decreased with exposure to ETS compared to FeNO levels during a non exposed period (24.7 ppb versus 18.7 ppb, p=0.003; Figure 3.3.9).

*Figure 3.3.10: Comparison in FeNO levels in children with asthma with low levels of ETS exposure and non ETS exposure*

This study also investigated the effects of low level ETS exposure (urinary cotinine: 10-30 ng/ml) on the FeNO levels in children with asthma. Nineteen children with asthma had changed from non ETS exposure to low ETS exposure between previous and later visits. This study found that FeNO levels were also decreased in children with asthma who recorded low levels of ETS exposure compared to a non ETS exposed period (23.4ppb versus 18.3 ppb, p=0.004; Figure 3.3.10).
Lung function

Figure 3.3.11: Changing in FEV$_1$ in children with asthma with and without ETS exposure

Twenty five children with asthma performed spirometry at both ETS and non ETS exposed periods. FEV$_1$ % predicted (SD) was 95.6% (10.8) during a non ETS exposed period and 90.8% (12.9) in an ETS exposed period. FEV$_1$ was lower when children were exposed to ETS (p=0.017), Figure 3.3.11.
The mean of FEV1/FVC ratio (SD) was 83.6 % (7.2) at a time without ETS exposure and 82.3% (8.8) during the ETS exposed period. No difference in FEV1/FVC ratio was found whether or not the children with asthma was exposed to ETS, (p=0.27), Figure 3.3.12.
Exhaled breath condensate

Twenty five children with asthma had EBC collected at both ETS exposed and non ETS exposed periods.

Figure 3.3.13: Comparison in pH of EBC of children with asthma with and without ETS exposure

The median pH (IQR) of EBC at a non ETS exposed period was 7.3 (7.1-7.5) compared to 7.1 (6.8-7.4) in an ETS exposed period. The pH of EBC in children with asthma during a non ETS exposed period tended to be higher than in an ETS exposed period (p=0.055).
**Sputum cell counts**

Sixteen children with asthma had sputum sample collected at both non ETS exposed and ETS exposed periods.

*Table 3.3.31: Induced sputum cell counts in children with asthma at with different ETS exposure periods*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children with asthma without ETS exposure</th>
<th>Children with asthma with ETS exposure</th>
<th>P value ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality (median, IQR)</td>
<td>18</td>
<td>17</td>
<td>0.42</td>
</tr>
<tr>
<td>Viability % median (SD)</td>
<td>88.1 (82-93.33)</td>
<td>71.8 (62.5-88.2)</td>
<td>0.04</td>
</tr>
<tr>
<td>TCC x 10^6/ml median (IQR)</td>
<td>3.96 (1.35-4.5)</td>
<td>1.71 (1.17-4.23)</td>
<td>0.28</td>
</tr>
<tr>
<td>Neutrophils % median (IQR)</td>
<td>20.75 (7.9-28.51)</td>
<td>26.5 (18.25-39.5)</td>
<td>0.04</td>
</tr>
<tr>
<td>Neutrophils absolute x10^4/ml, median (IQR)</td>
<td>48.26 ( 17.82- 62.88)</td>
<td>52.07 ( 30.35-113.15)</td>
<td>0.15</td>
</tr>
<tr>
<td>Eosinophils % median (IQR)</td>
<td>3.75 (0.25-13.75)</td>
<td>0.25 ( 0.25-3.5)</td>
<td>0.016</td>
</tr>
<tr>
<td>Eosinophil absolute x10^4/ml, median (IQR)</td>
<td>7.56 (2.88-31.68)</td>
<td>1.8(0.32-3.51)</td>
<td>0.006</td>
</tr>
<tr>
<td>Macrophage % median (IQR)</td>
<td>73.5 (38.75- 86)</td>
<td>69 ( 50.75-73.25)</td>
<td>0.5</td>
</tr>
<tr>
<td>Macrophage absolute x10^4/ml, median (IQR)</td>
<td>101.83 (66.4-436.05)</td>
<td>106.15 (65.34-311.85)</td>
<td>0.99</td>
</tr>
<tr>
<td>Lymphocyte % median (IQR)</td>
<td>1 (0-2)</td>
<td>0.5 (0-2.75)</td>
<td>0.77</td>
</tr>
<tr>
<td>Epithelial % median (IQR)</td>
<td>0.75 (0.13-1.75)</td>
<td>1.63 ( 0.38-4.25)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

‡ ‡ = Wilcoxon matched pairs test
The quality of sputum samples was similar whether children were exposed to ETS or not (17 versus 18, p=0.42). However, the viability of sputum cells in childhood asthma during a non ETS exposed period was significantly higher than at an ETS exposed period (88.1% versus 71.8%, p=0.04). In children with asthma, the total cell counts were $3.96 \times 10^6$/ml during a non ETS exposed period and $1.71 \times 10^6$/ml at an ETS exposed period, p=0.28.

*Figure 3.3.14: Sputum neutrophils in children with asthma with and without ETS exposure*

In children with asthma, % sputum neutrophils at that time they were not exposed to ETS was 20.75% compared with 26.5% at that time they were exposed to ETS. A significant increase in % sputum neutrophils in childhood asthma at ETS exposed period compared to non ETS exposed period was reported (p=0.04), Figure 3.3.14.
Figure 3.3.15: Comparison in absolute sputum neutrophils in children with asthma with and without ETS exposure

Only eleven children with asthma had enough sputum samples to analyze absolute neutrophil counts. In children with asthma, the number of sputum neutrophils (IQR) was $48.26 \times 10^4$/ml (17.82-62.88) at time non-exposure to ETS compared to $52.07 \times 10^4$/ml (30.35-113.15) at that time exposed to ETS. The number of sputum neutrophils was similar at both exposed points (p=0.15), Figure 3.3.15.
In subjects with asthma, the median % sputum eosinophil (IQR) was 3.75% (0.13-12.65) when they were not exposed to ETS. However, % sputum eosinophil was significantly decreased when they were exposed to ETS, down to 0.25% (0.23-2.63). It was a statistically significant difference in % sputum eosinophils between a non ETS exposure and ETS exposed period (p=0.016), Figure 3.3.16.
Eleven subjects had adequate sputum samples for analysis of absolute sputum eosinophils. There was a significant decrease in absolute sputum eosinophil counts at that time children with asthma were exposed to ETS compared to a non-exposed period (1.8 x 10^4/ml versus 7.56 x 10^4/ml, p=0.006) Figure 3.3.17.

No differences in sputum macrophages, lymphocytes, and epithelial cells were found in children with asthma with and without ETS exposure (p= 0.5, p= 0.77, p=0.22; respectively), Table 3.3.31.
Eleven children with asthma had sputum IL-8 results at both ETS and non ETS exposed periods. The median of sputum IL-8 (IQR) was 1.68 ng/ml (0.87- 2.05) at a non ETS exposed period compared to 1.58 ng/ml (1.02-9.02) at an ETS exposed period, p=0.17; Figure 3.3.18.
Table 3.3.32: Summary of the effects of ETS exposure on asthma markers

<table>
<thead>
<tr>
<th>Asthma markers</th>
<th>Non ETS exposure</th>
<th>ETS exposure</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma control score median (IQR)</td>
<td>0.29 (0-1.29)</td>
<td>0.42 (0.14-1)</td>
<td>0.33</td>
</tr>
<tr>
<td>FEV₁ % predicted mean (SD)</td>
<td>95.6 (10.8)</td>
<td>90.8 (12.9)</td>
<td>0.017</td>
</tr>
<tr>
<td>FEV₁/ FVC ratio %; mean (SD)</td>
<td>83.6 (7.2)</td>
<td>82.3 (8.8)</td>
<td>0.27</td>
</tr>
<tr>
<td>Exhaled NO ppb; median (IQR)</td>
<td>24.7 (9.8-47.8)</td>
<td>18.7 (9.4-31.4)</td>
<td>0.003</td>
</tr>
<tr>
<td>EBC pH median (IQR)</td>
<td>7.3 (7.1-7.5)</td>
<td>7.1 (6.8-7.4)</td>
<td>0.055</td>
</tr>
<tr>
<td>% Sputum eosinophils median (IQR)</td>
<td>3.75 (0.12-12.65)</td>
<td>0.25 (0.12-2.63)</td>
<td>0.016</td>
</tr>
<tr>
<td>Absolute sputum eosinophils x 10⁴/ml; median (IQR)</td>
<td>7.56 (2.88-31.68)</td>
<td>1.8 (0.32-3.51)</td>
<td>0.006</td>
</tr>
<tr>
<td>% Sputum neutrophils median (IQR)</td>
<td>20.75 (7.9-28.5)</td>
<td>26.5 (18.25-39.5)</td>
<td>0.04</td>
</tr>
<tr>
<td>Absolute sputum neutrophils x 10⁴/ml; median (IQR)</td>
<td>48.26 (17.82-62.88)</td>
<td>52.07 (30.35-113.15)</td>
<td>0.15</td>
</tr>
<tr>
<td>Sputum IL-8 (ng/ml) median (IQR)</td>
<td>1.68 (0.87-2.05)</td>
<td>1.58 (1.02-9.02)</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Table 3.3.33: Correlation between ETS exposure and pattern of airway inflammation

<table>
<thead>
<tr>
<th>Inflammatory pattern</th>
<th>Non ETS exposure</th>
<th>ETS exposure</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Eosinophilic asthma</td>
<td>57.1 (8)</td>
<td>42.9 (6)</td>
<td>0.75</td>
</tr>
<tr>
<td>% Non-eosinophilic asthma</td>
<td>51.9 (14)</td>
<td>48.1 (13)</td>
<td></td>
</tr>
</tbody>
</table>

The airway inflammatory pattern of children with asthma was divided into two subtypes upon the % sputum eosinophils. Children with asthma with sputum eosinophils more than 2.5% were classified as an eosinophilic pattern whereas sputum eosinophils equal or less than 2.5% were classified as a non-eosinophilic pattern. No difference in the distribution of airway inflammatory pattern in children with asthma with and without ETS exposure was observed (p=0.75, Table 3.3.33).
Discussion

This study has found that children who lived with smoking parents had a significantly higher risk to exposure to ETS compared with children living with non-smoking parents, especially if the parents smoked indoors. ETS exposure was high in all age groups of children with asthma. A change in ETS exposed pattern was associated with changes in airway inflammation. When compared to a non ETS exposed period, ETS exposure was associated with reduced FeNO levels, lower EBC pH, increased sputum neutrophils and decreased sputum eosinophils. There was also a reduction in lung function during ETS exposure. There results demonstrate the deleterious effects of ETS exposure on airway pathophysiology in children with asthma. These effects were seen in children with a varying ETS exposure pattern, and not seen in cross-sectional analyses.

Children, especially children with asthma, suffer from more health hazards when exposed to ETS. This study reported more than 30% of children with asthma living with parental smoking. Either mothers or fathers smoking can contribute to ETS exposure in children. Children were more likely to be exposed when both parents smoked, or smoking occurred inside the house.

1- ETS exposure in children with asthma

Environmental tobacco smoke exposure in children is very high. A survey in America in 2004 found that about 35% of all US children were exposed to ETS (554). An investigation of 4281 Australian children aged 0-4 years in 1989-1990 reported that 45% lived with at least one smoker, and 29% had a mother who smoked (555). ETS
exposure in children can be assessed based upon parental reports, exhaled carbon monoxide measurements and urinary cotinine.

1.1 -Parental reports

The prevalence of parent smoking varies from countries to countries. Almost all published papers report a correlation between parental smoking and the increased respiratory symptoms and respiratory diseases in their children. Passive smoking is known to be a major contributor to respiratory morbidity in children.

Environmental tobacco smoke exposure is one of the risk factors for the development of asthma in children. There is evidence linking both pre or post natal parental smoking, especially maternal smoking, and asthma development in children (263).

Parental smoking is frequent in children with asthma (556). The study by Mahabee-Gittens showed a large proportion of parents of children with asthma were smokers and their children’s asthma was exacerbated by cigarette smoke (410). Al-Dawook reported the smoking rate among parents of children with asthma was significantly higher than that of parents of healthy children (265). Other studies support that the parents of children with asthma were just as likely to be smokers as the parents of non-asthmatic children (557).

Although there is a strong link between ETS exposure and asthma development and exacerbation, a key question is whether parental smoking really results in ETS exposure in children. In a study in Turkey, ETS exposure was investigated in 188 school children based on parental reported smoking habits and children’s urinary cotinine. The study
found no correlation between parental reports and ETS exposure in children (558). Another study failed to find a relationship between parent’s reports of household smoking and ETS exposure in 4-16 year children (559). According to Karadag, parental questionnaires about smoking were unreliable in reporting ETS exposure in children with acute asthma (560).

However, an investigation of ETS exposure in three Western European countries including 347 German, 335 Dutch, and 354 Swedish preschool and schoolchildren by questionnaires for parents, air nicotine and urinary cotinine measurements in children indicated an increased ETS exposure in children with increasing questionnaire reported smoking (561). Parental smoking was a high predictor for infant exposure to tobacco smoke (562). Other studies have confirmed a significant correlation between smoking parents and positive urinary cotinine in their children (301). The urinary cotinine concentrations were higher in children living with smoking parents compared to children living with non-smoking parents (257, 563). My study agrees with these later reports that children with asthma who lived with smoking parents had a higher frequency of ETS exposure in comparison to children with asthma living with non-smoking parents. In fact, the prevalence of ETS exposure was highest in children with asthma who parents reported smoking inside (80%). However, there was only 5 parents reported smoking indoor, and the small number led to not enough power to detect the difference in ETS exposure between children living with and without parents smoking inside their houses. In group of parents reported smoking outside, prevalence of ETS exposure in children was 75%, and significantly increased compared to children living without parental smoking. The findings demonstrated that although parents reported only smoking outside,
their children still suffered from ETS exposure. In addition, whether parents reported smoking outside only was true, or they smoked both inside and outside. The data findings confirmed that children living with parents who smoked (both inside or outside) had higher risk to expose to ETS than children living with parents who were no smokers. However, in a group of children who lived with non parental smoking, about one third of children had positive urinary cotinine levels. The ETS exposure in this group may result from people other than parents who smoked such as the relatives, and guests... In the older children, they were likely exposed to ETS if their friends were smokers. Children may visit somewhere when smoking was permitted. Moreover, the question whether the parents were non-smokers was not confirmed.

Although there are some disagreements between parental reports and ETS exposure in children, the parent’s reports are still a reliable screen for ETS exposure in children.

1.2- Exhaled carbon monoxide measurement

Carbon monoxide is one of the toxic constituents in tobacco smoke. CO exists in the atmosphere as a product of incomplete combustion and oxidation of hydrocarbons. After inhalation, CO displaces oxygen in the erythrocyte to form carboxyhemoglobin(COHb), which has a half life of about 5-6 hours (273). CO is present in the exhaled air of normal people. Increased exhaled CO may occur in subjects who are exposed to environmental pollution, or are active or passive smokers. Measurement of exhaled CO in humans has been used as an indicator of smoking habit (564). Exhaled CO was significantly higher in
healthy smokers compared to nonsmokers (275). This study chose a cut-off level of 10 ppm to separate smokers with nonsmokers, as per previous guidelines (565).

Endogenous CO is produced in inflammatory processess which is the main pathogenetic mechanism of asthma, resulting in higher exhaled CO levels in subjects with asthma compared with healthy subjects. Exhaled CO levels were increased in children with asthma compared to healthy children (1.32ppm vs 0.86ppm, p=0.028) (566). A study demonstrated that children with persistent asthma had a significantly higher exhaled CO levels than children with infrequent episodic asthma (2.17 ppm vs 1.39ppm, p<0.05) (567), but no difference was found between infrequent episodic asthma and healthy children (567). By contrast, other studies did not find an elevation in exhaled CO levels in subjects with asthma (568, 569). According to Ohara, exhaled CO levels in infrequent episodic asthmatic children were not different from those in healthy children (1.1ppm vs 1ppm, p>0.68) (570). One study reported that exhaled CO levels were similar in asthma exacerbations, stable asthma and control subjects (571).

In addition, the difference was observed in the persistent asthma, but not in the infrequent asthma. Children with asthma did not receive ICS had significantly higher CO levels than those who received ICS (566, 572). Therefore, while exhaled CO can be elevated in asthma, the affect is not consistent for study to study, and the size of the effect is much smaller that seen with tobacco smoke exposure.

In this study, when parental smoking were not taken into consideration, the exhaled CO levels in children with athma [1ppm(1-1)] were similar in healthy children [1ppm (1-1)],
A comparison of children with asthma and healthy children of non-smoking parents, found that exhaled CO levels were also similar (1ppm vs 1ppm, p=0.49). These results may be explained by some reasons:

- Exhaled CO was measured by piCO Smokerlyzer Breath CO monitor, which recorded results as an ordinal number. Exhaled CO levels in children with asthma may be increased in comparison with healthy subjects, but the difference was very small (less than 1ppm) (566). Because piCO Smokerlyzer did not show the exhaled CO as a metric number, it may not have been sensitive enough to detect the difference of exhaled CO between healthy children and children with asthma.

- In this study, about 30% of children had persistent asthma but all of them were using ICS at the time of measuring exhaled CO, which resulted in no increased exhaled CO in children with asthma.

As a result of these reasons, endogenous CO may be less affected by asthma status.

Exhaled CO can also be used as an indirect measurement to assess passive smoking in children (281). Passive smoking is the major contributor to increased exhaled CO in healthy nonsmokers. Significant relationships were found between the numbers of cigarettes smoked in the house and an increase in exhaled CO levels in both healthy children and children with asthma who were non-smokers (281). In the current study, the median exhaled CO was 1ppm and no correlation between increased exhaled CO levels in children and parent reported smoking was found. This indicates that exhaled CO is not
a sensitive indicator to assess tobacco smoke exposure. The low levels of exhaled CO in subjects who were exposed to ETS can be explained by the short half life of exhaled CO (less than 5 hours). Children often participated in study early in the day or after school hours. If subjects were exposed to ETS by household smokers or visiting to smoking areas in the evening, exhaled CO may have returned to baseline by the next morning. Moreover, a free smoke environment is legislated in Australian schools, and as exhaled CO was measured during or after school hours, this may explain why exhaled CO was often at low levels. However, some children with asthma had high exhaled CO levels, and it can be explained by parents or carers smoking during transport of the children to their appointments. Parents and their children also had appointments on the vacation days, and children may be exposed to ETS from parental smoking or visitors who smoked at their homes or they may visit some smoking areas before going to the appointments.

1.3-Urinary cotinine

Cotinine is one of major metabolites of nicotine and can be measured to assess ETS exposure of non-smokers and reflects the degree of exposure (250). Compared with nicotine, cotinine has a longer half life, ranging 32-82 hours among non smoking children (287, 292). Cotinine levels in urine samples of non smoking children who were exposed to parental tobacco smoke at home were significantly higher than of children without parental smokers (573).
Although these are inconsistencies in the correlation between parents reported smoking and positive urinary cotinine in their children, children living with parental smokers have higher risk of exposure to ETS compared to children living with non-smokers (301, 574). Maternal smoking is reported to have a greater effect on children’s health than the paternal smoking, especially at high levels of consumption (301, 318). However, either mothers or fathers who smoked can contribute to children’s exposure to ETS (575) and affect on respiratory symptoms such as wheeze or cough (318, 576). The current study demonstrated a significant correlation between parents who smoked and ETS exposure in their children. Smoking mothers as well as smoking fathers had the same contribution to ETS exposure in the children, but the risk was significantly increased if both the father and mother were smoking.

High ETS exposure was found in children with asthma in this study. ETS exposure was high in all age groups. ETS exposure in the young children reflected the reported rate of parental smoking. On the other hand, ETS exposure was also high in the children in the 14 - 17 year group, where more than 50% of children in this age group had positive urinary cotinine results. The older children could be exposed to ETS in their homes by household smokers or by visiting public areas where smoking was permitted or by their friends who smoked. Alternatively, the cotinine levels in older children may reflect as intermittent pattern of active cigarette consumption. We assume that the young children were basically exposed to ETS from their parents whereas older children were exposed to ETS by household smokers or the environment. However, because of the missing in
urine collection at the visit 1 (only 62\% urine samples were collected), the data analysis may be a possibly bias.

2-Clinical characteristics

+ Age of asthma diagnosis

This study found that more than 60\% of childhood asthma was diagnosed before 2 years of age in the group without parental smoking compared to 35\% of children with asthma with parental smoking. Previous studies reported that in utero exposure to tobacco smoking have a stronger effect on the onset of asthma in children (262, 263). A study in Sweden found that ETS exposure during childhood may affect the prevalence of asthma in adults, suggesting that children living with smoking parents may have a later onset of asthma. Children living with smoking parents may have decreased GP visits or health care contacts, which may result in the later diagnosis of asthma in children (266).

+ Asthma symptoms

Several studies did not find any correlation between passive smoking and asthma symptoms in children. Karadag et al reported no difference in the frequency of passive smoking exposure in children with asthma in acute periods compared with asymptomatic periods (560). Forbes et al found no evidence for increased emergency visits in children with asthma by passive smoking exposure in the houses (577).
However, most studies report that ETS exposure in children with asthma was associated with increased asthma symptoms (133, 550) exacerbations (250, 337) and frequent hospitalization due to asthma attacks (250). Sturm et al. reported that even very low levels of ETS exposure also induced asthma symptoms (133).

Either mothers or fathers who smoked can affect respiratory symptoms such as wheeze or cough (318). However, mothers who smoke have a stronger effect on asthma symptoms in children. Children were more likely to wheeze if their mothers smoked (256, 260). A significant correlation was also reported between maternal smoking and the frequency of wheeze in the last 12 months (578). Interestingly, female adolescents whose mothers had smoked during pregnancy and the early months of life had increased the asthma symptoms during adolescence (262). High levels of ETS exposure were also related to increased nocturnal symptoms in asthma (OR: 2.83; 95% CI: 1.22-6.55)(336). In addition, smoking in the home by people other than parents was also significantly associated with increased asthma symptoms (OR: 1.49; 95% CI: 1.13-1.97) (134).

Raised urinary cotinine levels were related to increased asthma exacerbations among children (257, 337). Children who had asthma and whose parents smoked have more frequent asthma attacks and more severe symptoms (334, 337). Children living with current smoking family members had significantly increased hospital visits due to asthma worsening (579). The recovery period after hospitalization for an asthma attack was impaired if children were exposed to ETS, characterized by persistent respiratory symptoms and often use of relief medication (340).
Therefore, although some studies reported no evidence for an association between asthma symptoms and passive smoke exposure, most studies agreed that passive smoking caused increased symptoms and exacerbations in children with asthma. The disagreement between studies may be explained by the difference in the numbers of subjects of each study. The larger, epidemiological studies are needed to regularly see an effect of ETS on asthma. In addition, passive smoking is only one of several asthma triggers, and asthma symptoms or exacerbations may be induced by a single or multiple triggers, which makes it difficult to determine the main factor causing asthma symptoms.

+ Asthma control score

Smokers with asthma have poor control of symptoms, worse quality of life (551, 580) and an accelerated rate of decline in lung function (551), which explains an increase in asthma control score compared to non-smokers with asthma.

An increased asthma morbidity, poor asthma control and lower quality of life were also reported in asthmatic children with ETS exposure (334). Asthma score was related to passive smoking exposure (581). These findings may explain the increased asthma control score and the decreased FEV\textsubscript{1} seen in this study when children with asthma who were exposed to ETS compared to a non ETS exposed period.

My study also reported that the asthma control score in children living with smoking parents was higher than children living with non-smoking parents, and also that during a non ETS exposed period, children with asthma had a lower asthma control score compared to an ETS exposed period. While the results failed to reach statistical
significance, they suggested that children living with parental smoking or who are exposed to passive smoking may have poor asthma control compared to children living with non-smokers or not exposed to ETS. However, ACS using in this study has been validated to adults, when the questionnaire was applied to children, it may have a possible bias. The asthma symptoms of young children in the last week was reported by the parents and was subjective by parent feelings. A questionnaire about ACS which is appropriate for children should be designed.

+ Clinical pattern

Current smoking has been associated with an increased severity of asthma (92, 310, 582). Eisner et al assessed the effects of passive smoking in adults with asthma, and found that greater asthma severity was related to the highest levels of ETS exposure (583). Exposure to ETS was also linked to impaired lung function, and increased airway obstruction (584). Consistent with this, my study found that the prevalence of parental smoking was higher in frequent and persistent childhood asthma compared to infrequent asthmatic children. In the persistent asthma group, 40% of children lived with at least one smoker in their home.

According to DiFranza, children who were exposed to ETS had decreased lung growth and increased respiratory symptoms, and the severity of symptoms increased with increased exposure (585). Tobacco smoke exposure were positively associated with asthma symptom severity (586). Halterman et al reported that nearly half of the children with persistent asthma lived with at least one smoker (587). Asthma severity in children, which required frequent emergency visits because of the presence of current
smoking family members, was described (579). Children with asthma who lived with smoking parents commonly suffered from increased asthma symptoms, respiratory infections, acute episodes and frequent hospitalization (92).

+ Treatment

Few studies currently investigate the impact of smoking on asthma treatment. Recent data showed strong evidence of corticosteroid resistance in smokers with asthma for both short-term or long-term, and low dose or high dose treatment (313, 378, 379). In addition, the response to ICS was even decreased in smokers with mild asthma (580).

The recovery period after hospitalization for an asthma attack was impaired if children were exposed to ETS, characterized by persistent respiratory symptoms and frequent use of relief medication (340). The present study reported that the doses of ICS taken by children with asthma who lived with smokers were higher than those did not live with smokers. However, the number of subjects in this study was small, and ICS use may affect on airway phenotype. A larger study can address to validate the findings. To my knowledge, no published papers have reported the effects of passive smoking on ICS used in children with asthma. More investigation is needed in order to differentiate the corticosteroid requirements between childhood asthma with and without ETS exposure.

According to Crombie, high levels of parental smoking were related to a decrease in scheduled visits to GP or hospital for asthma management in children (266). Also a high frequency of children with undiagnosed asthma was related to passive smoking
exposure (588). The findings suggest that children who live with smokers may not receive adequate health care services and treatment (266).

3- **Atopy**

In healthy children, there was no reported difference in the prevalence of atopy in children with and without parents who smoked (589). A study of about 9000 children in the four or fifth grades also showed no association between parental smoking and atopy in children (590), and in other words, passive smoking exposure had little or no effect on the development of atopy in children (96, 591). However, Larsson reported that paternal smoking, but not maternal smoking was a significantly risk for the development of atopy in children (592). Another study found a higher risk for developing atopic eczema in children when exposed to ETS (593). A review by Strachan and Cook of 1593 papers found that parental smoking, either before or after birth, was inconsistently related to the risk of allergic sensitization (pooled odds ratio:0.87, 95% CI: 0.62-1.24).

According to Cantani, passive smoking was significantly associated with the development of asthma in atopic children (256). Moreover, cigarette smoking was also a risk factor for asthma development in non-atopic children (256). A survey of Canadian children reported that there was an association between parental smoking and recent asthma among children without a history of atopy (OR: 2.93, 95% CI: 0.83-10.3) rather than among children with history of asthma (OR: 0.73, 95% CI: 0.37-1.46) (332).
Current cigarette smoking may decrease FeNO levels in both healthy subjects and subjects with asthma (360-362). Kharitonov et al found that smoking only a single cigarette transiently and immediately reduced NO (361). Cigarette smoking can inhibit iNOS, which results in the reduced FeNO levels. Decreased iNOS protein and mRNA expression in the airway epithelial cells had been found in smokers (361, 594). The effects of cigarette smoking on iNOS may be explained by a inhibitory feedback mechanism that results from the high levels of exogenous NO that are released by smoking (363), or by the carbon monoxide in the cigarette smoke that can inhibit iNOS (364). Cigarette smoking may also modify airway inflammation in subjects with asthma. Induced sputum studies show increased neutrophils and suppressed eosinophils in smokers with asthma (314, 348, 595), suggesting that the lower FeNO levels may also be due to a non-eosinophilic pattern of inflammation.

There are few studies investigating the effects of passive smoking on FeNO levels. Experiments in healthy adult subjects who were exposed to smoke over a period of 1 hour showed a rapid decrease in FeNO levels that remained low for 60 minutes (366). Franklin et al failed to find any difference in FeNO levels between children who lived with and without a smoker (453). In children with asthma, Warke found an association between parental reported ETS exposure in children and lower FeNO levels (367). Barreto et al assessed ETS exposure in children with asthma based on questions about daily cigarette consumption at home by the parents or other household members and found no difference in FeNO levels in childhood asthma with or without ETS exposure.
(368). Dinakar et al compared FeNO levels in children with asthma with and without ETS exposure based on nicotine/cotinine urine, and again no difference in FeNO levels was found (369). My study using urinary cotinine test to determine ETS exposure in children with asthma also did not find decreased FeNO levels in children with asthma who were exposed to ETS compared to those without ETS exposure. FeNO levels were also similar in children with asthma living with and without parental smokers. These cross-sectional studies are consistent in failing to detect the effect of smoking on FeNO. I suggest that the sample sizes of my study were small, that may be not enough power to detect the effect of passive smoking on FeNO in these cross-sectional studies. A comparison of FeNO levels in children with asthma at different ETS exposed levels found that FeNO levels in children with high ETS exposure were 10 ppb lower than children without ETS exposure. However, the number of children with asthma who had high ETS exposure was small (n=5), this resulted in failure to detect the difference. Decreased FeNO in children with asthma who were exposed to high ETS may be intermediate between those without ETS exposure and smokers. When I compared FeNO levels in each child with asthma between an ETS exposure and a non ETS exposure, a significant decrease in FeNO levels in children with asthma during ETS exposure was found. Moreover, FeNO levels in children with asthma were decreased by even a very low level of ETS exposure. This suggests that FeNO levels in children with asthma were decreased when exposure to ETS is compared to their baseline (no ETS exposure). FeNO levels are high in children with asthma compared to healthy children. However, increased FeNO levels are different from subject to subject. In this study, FeNO levels were reduced about 6ppb when children with asthma were exposed to ETS,
but those were decreased only when compared to themselves at a non ETS exposure. The finding may explain the difficulty to detect the effect of passive smoking on FeNO levels when conducting cross-sectional studies between unpaired groups because of different increased FeNO levels in children with asthma. The mechanism of lower FeNO levels in children with asthma who were exposed to ETS may be similar to the mechanism of decreased FeNO levels in active smokers. These results are consistent with controlled exposure studies (361, 366) that show reduced FeNO with tobacco smoke exposure.

5- Lung function

Both in utero exposure to maternal smoking and postnatal ETS exposure are associated with persistent deficits in lung function in healthy children and children with asthma (334, 335, 596). Ferris et al found that FEV$_1$ was lower in children who lived with smoking mothers compared to children of nonsmoking mothers (597). A study in 571 children aged 8-16 years found that children who lived with smoking fathers had a 0.5% decreased FEV$_1$ compared to FEV$_1$ predicted values (598). A prospective study estimated that if two children have the same demographic characteristics but one lived with smoking mother and another had not, the reductions in % FEV$_1$ over time of the child with a smoking mother were 10.7%, 9.5% and 7% after one, two and five years, respectively compared with the child who lived with a non smoking mother (599). These results suggest that passive exposure to tobacco smoke may have important effects on the development of pulmonary function in children.
Children with asthma who had smoking mothers suffered from more severe asthma symptoms and impaired lung function than children of non-smoking mothers (600). The more ETS exposure in children with asthma, the lower their lung function (337). The current study did not find the difference in FEV$_1$ % predicted in the children with asthma living with and without parental smokers, and in the children with asthma with and without ETS exposure in these cross-sectional studies. However, there was a significantly decreased FEV$_1$ in children with asthma during a period of ETS exposure compared to non-ETS exposed period in the longitudinal study. This is consistent with an adverse effect of ETS on lung function in children with asthma. The difficulty to detect the effects of passive smoking on lung function in children with asthma in these cross-sectional studies may be explained by the small number of the subjects in these studies. In addition, the FEV$_1$ was reduced at an ETS exposure compared to those at a non-ETS exposed period in the paired comparison. When these cross-sectional studies are performed in the unpaired groups, a larger number of study subjects should be recruited to have enough power to detect the effects of passive smoking on lung function in children with asthma.

6-Airway hyperresponsiveness

AHR is quite common in smokers compared to non-smokers, and significantly decreased after smoking cessation (601). Willemse reported that a year after smoking cessation led to a remarkable improvement in both direct and indirect AHR (602). Another study showed that stopping smoking led to improved respiratory symptoms and FEV$_1$ % predicted but no change in AHR (603).
Passive smoking is associated with the development of asthma. Experimental studies in animal models showed prenatal ETS exposure led to increased airway hyperresponsiveness. However, exposure of adult mice to cigarette smoke did not significantly alter airway responsiveness (604). In humans, the effects of passive smoking on AHR are not been well established. A study in 503 children 10-12 years did not find any difference in AHR whether parents smoked or not (589). Joad reported that prenatal ETS exposure from the mother caused a deficit in fetal lung development, airway obstruction and AHR (605). Cook and Strachan reported that the pooled odds ratio of having AHR in school aged children exposed to maternal smoking was 1.29 (95% CI: 1.1-1.5) (374). This indicates a small but significant effect of ETS on AHR. A review of the papers which were published in Thorax in 1999 suggested that both ETS exposure from in-utero and posnatally could possibly contribute to bronchial hyperresponsiveness in children (96). Also bronchial responsiveness and airway sensitization to inhaled stimuli may also increase in asthmatic subjects if they were exposed to ETS (250, 550).

Sputum eosinophils are associated with the degree of AHR in subjects with asthma (178, 508). Subjects with non- eosinophilic asthma had less severe AHR compared to subjects with eosinophilic asthma (191). In this study, children with asthma had lower sputum eosinophils at ETS exposed period compared to non ETS exposed period. This finding may explain the decrease in the proportion of AHR in children with asthma living smoking parents compared to those living with non smokers, and the lower proportion of AHR in children with positive urinary cotinine compared to those with negative urinary cotinine.
7- **Exhaled breath condensate**

Active cigarette smoking induces an inflammatory response, including increased sputum neutrophil counts and more acidity of exhaled breath condensate pH compared to non-smokers (595). An experimental study in smokers found that after smoking 2 cigarettes, the level of nitrate in EBC was increased (606), suggesting the lower EBC pH.

Currently, no publications have reported the effect of passive smoking on EBC pH. My study demonstrated that EBC pH in children with asthma during an ETS exposed period was lower compared to a non ETS exposed period. This suggests that passive smoking exposure may induce an inflammatory process in asthma, which results in more acidity of the airways, and lower EBC pH compared to a non ETS exposed period.

8- **Airway inflammation**

A fundamental question is whether smoking induces a distinct airway phenotype of asthma compared with non smoking. Cigarette smoking induces airway inflammation in normal smokers, with the elevated neutrophil numbers (343), T- lymphocytes (mainly CD8) (344) and macrophage numbers (343). There are few data on the potential influence of smoking on asthmatic airway inflammation. Cigarette smoking may modify airway inflammation in subjects with asthma, leading to a the predominance of non-eosinophilic inflammation (311). An experimental study in animal models showed that cigarette smoking may induce neurogenic inflammation (345), elevated oxidative stress (346) which leads to an increase in cysteinyi leukotrienes, and an amplification of the airway
inflammation in subjects with asthma (347). Reduced sputum eosinophils and elevated sputum neutrophils have been observed in asthmatic adult smokers (348).

In summary, active smoking can alter airway inflammatory cells in adults with asthma, includes increased neutrophils and/or reduced eosinophils (314). The mechanism of the difference in airway inflammation in asthmatic smokers compared to asthmatic non-smokers is not clear. The reduced sputum eosinophils in smokers with asthma may be explained due to an increase in eosinophil apoptosis by toxic components in tobacco smoke (349). Some substances deriving from cigarette smoke such as CO may also attenuate airway eosinophils (350).

Not only active smoking, but passive smoking has been linked to altered inflammatory phenotypes in animal models. A study in mice exposed to ETS in the short term found reduced BAL eosinophil numbers in comparison to mice without ETS exposure (365). If male Wistar rats were exposed to ETS every 2 hours per day for 2 weeks, increases in the numbers of neutrophils, lymphocytes, and macrophages in BAL in twenty four hours after the last cigarette smoke exposure were observed (607).

To my knowledge, no publications currently describe the effects of passive smoking on airway inflammation in humans with asthma. In these currently cross- sectional studies, both % sputum eosinophils and absolute sputum eosinphils were higher whereas the absolute sputum neutrophils were lower in children with asthma living with non-smokers compared to children with asthma living with smokers, but these differences were not
statistical significance. Similarly, there were no differences in sputum eosinophils and neutrophils in children with asthma with and without ETS in a cross-sectional study. However, a longitudinal study found that both the percentages of sputum eosinophils and absolute sputum eosinophils were decreased in children with asthma who were exposed to ETS. In addition, the percentages of sputum neutrophils were elevated when children with asthma were exposed to ETS compared to non-ETS exposed period. The different findings between the cross-sectional studies and a longitudinal study may be explained by the number of study’s subjects and the investigated methods. In the longitudinal study, I investigated and compared sputum cell counts in the paired group, that means the results were assessed in the same subjects at the different time, and the investigation was not required to many subjects. However, in these cross-sectional studies, I investigated and compared sputum cell counts in the unpaired groups, and each subjects in this study had different sputum cell count levels, which required the larger number of study’s subjects in order to assess the difference between the different groups. Cigarette smoke can induce the release of IL-8 (608, 609) and TNF-alpha (608, 610). The pro-inflammatory cytokines such as IL-8 and TNF-alpha were significantly increased in smokers in comparison with non-smokers. After smoking cessation, both IL-8 and TNF-alpha were significantly decreased (611). IL-8 and TNF-alpha are known as potent chemoattractants for neutrophils, and they can induce transepithelial migration of neutrophils. In vitro, experiments report that nicotine stimulated neutrophil-IL-8 production via nicotinic acetylcholine receptors, results in NF-kappa B activation, thereby producing IL-8 (612). IL-8 release can attract neutrophils to the airway.
The mechanisms of reduced sputum eosinophils and elevated sputum neutrophils by ETS exposure may be similar to those smokers with asthma. Heterogeneity of airway inflammation where many cells take part is now recognized as a feature of asthma. There are at least two subtypes of airway inflammation that have been classified, one with increased sputum eosinophils and increased FeNO levels, and another with normal sputum eosinophils and normal FeNO levels. Passive smoking may modify airway inflammation in children with asthma, with reduced FeNO levels, lower EBC pH and decreased sputum eosinophils, leading to a non-eosinophilic pattern. However, subjects with asthma with non ETS exposure also showed a non-eosinophilic pattern. The findings suggest that there is more than one mechanism that leads to the development of non-eosinophilic airway inflammation, and passive smoking is one of the factors modify airway inflammation in subjects with asthma.

**Conclusion**

In conclusion, smoking is a hazard not only for smokers, but also for people who are exposed to passive smoke, especially children. The prevalence of ETS exposure in childhood asthma is still high. Children with asthma living with parents who smoke seem to have more severe asthma compared with children living with non-smokers.

ETS exposure in children with asthma can modify airway inflammation, with decreased FeNO levels, lower EBC pH, reduced sputum eosinophils and elevated sputum neutrophils. Asthma in children who were exposed to ETS was associated with non eosinophilic pattern of airway inflammation.
CHAPTER IV

KNOWLEDGE AND ATTITUDES OF PARENTS OF CHILDREN WITH ASTHMA TOWARDS PASSIVE SMOKING

Introduction

The harmful effects of active smoking are now well known. Although many people are aware of the health dangers of smoking, these dangers are frequently underestimated. According to Gun et al., if people smoke more than 30 cigarettes per day, they have a risk of more than a threefold increase in all-cause mortality, a 60% increase in cancer incidence, a 43-fold increase in lung cancer incidence, and a more than fourfold increase in mortality from ischaemic heart disease (613).

Passive smoke contains the same toxic substances as identified in mainstream tobacco smoke. Non smokers who are exposed to ETS may suffer from the same health hazards as active smokers. Parental smoking is strongly associated with ETS exposure in their children (301, 614). In addition, smoking parents are more likely to recruit their children to become smokers (408).

Both active and passive smoking are significantly correlated with asthma development and asthma exacerbation in children. Australia is one of the countries with the highest asthma prevalence in the world. The prevalence of Australian children living with smokers is also high. According to the ABS, in 2001 38% children under 12 years lived in homes with at least one adult who was a regular smoker (255). Previous studies
reported that the smoking rate among parents of children with asthma was significantly higher than that of parents of healthy children (265). We questioned whether the parents of children, especially children with asthma, knew the effects of passive smoking on their children’s health, and whether they knew how to avoid ETS exposure in their children.

**Aims**

This study aimed to investigate the knowledge and the attitudes of parents of children with asthma towards passive smoking. The study also assessed whether nicotine addiction may influence the attitudes of parents to their smoking habit.

**Hypotheses**

The study tested the hypotheses that parents of children with asthma understood the harmful effects of ETS exposure in children, especially children with asthma, and that smoking parents knew how to avoid ETS exposure in their children. However, the smoking cessation was difficult because it depended on many other factors such as smoking habits, the level of addiction, and the level of knowledge towards passive smoking.
Methods

Study design

This cross-sectional study was conducted in two cities: Newcastle (Australia) and Hanoi (Vietnam). In Australia, one parent of each child with asthma who accompanied their child to study visits was invited to participate in this study at one time between visit 1 and visit 3. In Hanoi, one parent of a child with asthma was invited to attend for one visit. Parents were asked a series of questions about their knowledge and attitudes towards passive smoking. Nicotine addiction was also assessed by questionnaire if the parent was a smoker. Urine samples from children with asthma were collected at this visit to assess ETS exposure in the children.

Study subjects

One parent who accompanied their child at the study visit was invited to participate in this study at one visit. Parents and children with asthma in Hanoi were recruited from the Outpatient clinics at the Pediatric department, BACH MAI Hospital (Hanoi, Vietnam).

Children with stable asthma that were diagnosed by paediatricians based on clinical and lung function criteria in both Newcastle and Hanoi were asked to provide a urine sample during a visit. The change in FEV₁ after bronchodilator used was performed at visit 2 and visit 3. All children with asthma in Newcastle were aged between 7-17 years and children with asthma in Hanoi were aged less than 15 years.
**Measurements**

*Questionnaires*

In this study, the questionnaires were designed in four sections (Appendix V).

In section I, the parent was asked a series of questions about smoking status, including who smoked, a history of smoking: never smoker, ex-smoker or current smoker, the number of cigarettes smoked per day, where they smoked: inside or outside, in the car etc…and estimated ETS exposure on children. The questionnaires were modified from previous publications (300, 404, 410).

Section II was performed if the parent was a smoker. The level of nicotine addiction was assessed using the Fagerstrom questionnaire (409).

In section III, the parent was asked a series of questions to assess their knowledge and their attitudes towards passive smoking. The questions were modified from a previous publication (408).

The questions in section IV were asked if the parent was a smoker. These questions evaluated whether the parent wanted to quit smoking and whether they knew to avoid or limit ETS exposure in their children.
Urine collection

Urine samples were collected from the children in order to assess their exposure to environmental tobacco smoke.

Measurement of cotinine in urine

Children’s urine was collected for measurement of urinary cotinine using Nic-Alert test strips (Nymox Pharmaceutical Corporation, Maywood, NJ, USA). Nic-Alert tests are used to identify active smoking as well as to detect passive smoking exposure. ETS exposure was defined as urinary cotinine in range 10-100 ng/ml (437). The procedure of urinary cotinine measurement has been described in Methods chapter.

Statistical methods

Statistical analysis was carried out using STATA (STATA Corporation, College Station, Texas, USA). Characteristics of the study population and parametric results were expressed as geometric mean and standard deviation (SD). Non parametric results were reported as median and interquartile range (IQR). Group comparisons were conducted. For variables with normal distribution, Students t-test was performed. Nonparametric data was analyzed by the Mann-Whitney test for two groups. Chi-squared test and Fischer’s exact test was used for categorical data. A p < 0.05 was accepted as statistically significant.
Results

There were 47 Australian parents and 72 Vietnamese parents who agreed to participate in this study.

Table 3.4.1: Characteristics of parents of children with asthma in Newcastle and Hanoi

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Newcastle (Australia)</th>
<th>Hanoi (Vietnam)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>47</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td><strong>Age (Mean, SD)</strong></td>
<td>40.5 ± 7.9</td>
<td>35.9 ± 9.9</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>Gender (% female)</strong></td>
<td>95.74</td>
<td>71.83</td>
<td>0.001$</td>
</tr>
<tr>
<td><strong>Marital Status:</strong></td>
<td></td>
<td></td>
<td>0.016$</td>
</tr>
<tr>
<td>Single</td>
<td>2.13%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>80.85%</td>
<td>97.14%</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>4.26%</td>
<td>1.43%</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>12.77%</td>
<td>1.43%</td>
<td></td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td>0.001$</td>
</tr>
<tr>
<td>Primary school</td>
<td>0%</td>
<td>14.3%</td>
<td></td>
</tr>
<tr>
<td>Secondary school</td>
<td>36.17%</td>
<td>28.6%</td>
<td></td>
</tr>
<tr>
<td>TAFE</td>
<td>34.04%</td>
<td>11.4%</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>29.79%</td>
<td>45.7%</td>
<td></td>
</tr>
<tr>
<td><strong>Employment Status</strong></td>
<td></td>
<td></td>
<td>0.0001$</td>
</tr>
<tr>
<td>Full time</td>
<td>21.28%</td>
<td>82.86%</td>
<td></td>
</tr>
<tr>
<td>Part-time</td>
<td>51.06%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>25.53%</td>
<td>17.14%</td>
<td></td>
</tr>
<tr>
<td>Student</td>
<td>2.13%</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>
The demographic characteristics of parents of children with asthma in Newcastle and Hanoi are described in Table 3.4.1. The parents of children with asthma in Hanoi were younger than the parents of children with asthma in Newcastle (p=0.001). The questionnaires were completed predominantly by mothers of children with asthma in the two cities. The proportion of mothers in Newcastle participating in this study was significantly higher than those of mothers in Hanoi (95.74% versus 71.83%, p=0.001). The marital status was different between parents in the two cities, with about 81% of parents in Newcastle and 97% of parents in Hanoi married (p=0.016). While all parents in Newcastle had graduated at least secondary school, 14.3% of parents in Hanoi graduated primary school (p=0.001). Employment was also different, 82.86% of parents in Hanoi working full-time compared to 21.8% in Newcastle, where 51% of parents worked part-time. The differences in demographic characteristics between the parents of children with asthma in the two cities can be explained by the differences in the culture and society between Australia and Vietnam.
Table 3.4.2: Characteristics of ETS exposure in children with asthma in Newcastle and Hanoi

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Newcastle (Australia)</th>
<th>Hanoi (Vietnam)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% reported children living with smoking parents</td>
<td>30.3%</td>
<td>49.28%</td>
<td>0.023*</td>
</tr>
<tr>
<td>Number of cigarettes daily, median (IQR)</td>
<td>15 (3-30)</td>
<td>20 (1-20)</td>
<td></td>
</tr>
<tr>
<td>Estimated ETS exposure in children per week, hour; median (range)</td>
<td>0 (0-7)</td>
<td>0.5 (0-7)</td>
<td></td>
</tr>
<tr>
<td>% children who were exposed to ETS (urinary cotinine tests)</td>
<td>46.3%</td>
<td>24.3%</td>
<td>0.017*</td>
</tr>
</tbody>
</table>

* = Pearson Chi² Test

About 30% children with asthma in Newcastle lived with smoking parents compared to 49.28% of children with asthma in Hanoi, p=0.023. However, when assessing ETS exposure 46.3% of children with asthma in Newcastle had a positive urinary cotinine tests compared to 24.3% of children with asthma in Hanoi (p=0.017). Interestingly, while we cannot find any correlation between parental smoking and ETS exposure in children with asthma in Hanoi (p=0.95), there was a statistically significant correlation between smoking parents and ETS exposure in their children in Newcastle (p=0.029).

The parent who smoked was also different between the two cities. In Newcastle, 45% of fathers reported to be smokers, while maternal smoking was 25%, and both mothers and fathers smoked in 30% of families. However, 100% of parents who smoked in Hanoi were fathers. Different parental contact and smoking pattern may explain the lower prevalence of positive urinary cotinine levels in Hanoi.
The level of Nicotine addiction of smoking parents of children with asthma

Fagerstrom et al have classified five levels of nicotine addiction using a 6-question scale: very low (0-2), low (3-4), medium (5), high (6-7), and very high (8-10) (409). We interviewed 9 (19.1%) smoking parents of children with asthma in Newcastle and 11 (15.3%) smoking parents of children with asthma in Hanoi to assess the level of nicotine addiction.

Figure 3.4.1: Distribution of Fagerstrom test for Nicotine Dependence (FTND) scores of smoking parents of children with asthma in Newcastle.
The levels of nicotine addiction of smoking parents of children with asthma in the two cities are described in Figure 3.4.1 and Figure 3.4.2. The data shows that 77.8% of smoking parents of children with asthma in Newcastle and 54.6% of smoking parents of children with asthma in Hanoi had very low or low levels of nicotine addiction. On the other hand, 22.2% of smoking parents of children with asthma in Newcastle and 27.3% of smoking parents of children with asthma in Hanoi had high or very high levels of nicotine addiction. The levels of nicotine addiction was similar in the parents of children with asthma in the two cities (p=0.55).
Knowledge and attitudes of parents of children with asthma about passive smoking

Table 3.4.3: Knowledge of parents of children with asthma about passive smoking

<table>
<thead>
<tr>
<th>Passive smoking causes (% subjects)</th>
<th>Newcastle (Australia)</th>
<th>Hanoi (Vietnam)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Cancer</td>
<td>97.87</td>
<td>98.5</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>29.79</td>
<td>11.9</td>
</tr>
<tr>
<td>Bowel Cancer</td>
<td>29.79</td>
<td>22.3</td>
</tr>
<tr>
<td>Brain Tumor</td>
<td>29.79</td>
<td>35.8</td>
</tr>
<tr>
<td>Erection problems</td>
<td>36.17</td>
<td>62.7</td>
</tr>
<tr>
<td>Heart attacks</td>
<td>78.72</td>
<td>82.1</td>
</tr>
<tr>
<td>Stroke</td>
<td>74.47</td>
<td>49.3</td>
</tr>
<tr>
<td>Asthma attacks</td>
<td>97.87</td>
<td>98.5</td>
</tr>
<tr>
<td>Emphysema</td>
<td>91.49</td>
<td>82.1</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>23.4</td>
<td>28.4</td>
</tr>
<tr>
<td>Blindness</td>
<td>21.28</td>
<td>6.0</td>
</tr>
<tr>
<td>Deafness</td>
<td>4.26</td>
<td>4.5</td>
</tr>
<tr>
<td>Cataracts</td>
<td>17.02</td>
<td>19.4</td>
</tr>
<tr>
<td>Skin Cancer</td>
<td>14.89</td>
<td>28.4</td>
</tr>
<tr>
<td>Arthritis</td>
<td>8.51</td>
<td>13.4</td>
</tr>
<tr>
<td>Smaller babies</td>
<td>87.23</td>
<td>86.6</td>
</tr>
<tr>
<td>Lower levels of intelligence in children</td>
<td>40.43</td>
<td>83.6</td>
</tr>
<tr>
<td>Ear infection</td>
<td>17.02</td>
<td>40.3</td>
</tr>
<tr>
<td>Tonsillitis in children</td>
<td>17.02</td>
<td>70.1</td>
</tr>
<tr>
<td>Meningococcal diseases in children</td>
<td>6.38</td>
<td>26.9</td>
</tr>
<tr>
<td>Eczema in children</td>
<td>12.77</td>
<td>25.4</td>
</tr>
<tr>
<td>Fainting</td>
<td>25.53</td>
<td>46.3</td>
</tr>
<tr>
<td>SIDS (Sudden infant death syndrome)</td>
<td>74.4</td>
<td>49.3</td>
</tr>
</tbody>
</table>
All parents of children with asthma in both Newcastle and Hanoi agreed to answer the survey. If more than 50% of parents said “Yes” for each item, we considered that most parents knew that the diseases may be caused by passive smoking. Table 3.4.3 shows that parents of children with asthma in both Newcastle and Hanoi agreed that passive smoking is known to cause lung cancer, heart attacks, asthma attacks, emphysema, and smaller babies. Parents in Newcastle noted that passive smoking also caused stroke and SIDS whereas parents in Hanoi indicated that passive smoking caused erection problems, lower levels of intelligence in children, and tonsillitis in children.

Table 3.4.4: Attitudes of parents of children with asthma towards passive smoking

<table>
<thead>
<tr>
<th>Attitudes ( % subjects)</th>
<th>Newcastle (Australia)</th>
<th>Hanoi (Vietnam)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults have a right to smoke wherever they want in their own homes</td>
<td>23.4%</td>
<td>2.9%</td>
<td>0.001  *</td>
</tr>
<tr>
<td>Children should have the right to live in a smoke-free home.</td>
<td>97.87%</td>
<td>94.2%</td>
<td>0.32    *</td>
</tr>
<tr>
<td>An act should be passed which forbids all indoor smoking in the vicinity of children.</td>
<td>97.87%</td>
<td>89.86%</td>
<td>0.093  *</td>
</tr>
<tr>
<td>Children who are exposed to ETS are more likely to start to smoke themselves</td>
<td>44.68 %</td>
<td>63.77%</td>
<td>0.042  *</td>
</tr>
<tr>
<td>Children who are exposed to ETS are more likely to develop asthma</td>
<td>70.21 %</td>
<td>89.86%</td>
<td>0.007  *</td>
</tr>
<tr>
<td>Children who are exposed to ETS are more likely to have asthma attacks</td>
<td>78.72%</td>
<td>88.4%</td>
<td>0.16    *</td>
</tr>
</tbody>
</table>
While 23% of Newcastle parents agreed that “Adults have a right to smoke wherever they want in their own homes”, only 3% Hanoi parents agreed with this statement, \( p=0.001 \). However, most parents of both cities (\( \geq 90\% \)) have agreed that “Children should have the right to live in a smoke-free home” and “An act should be passed which forbids all indoor smoking in the vicinity of children” (\( p=0.32, p=0.093 \); respectively). In Newcastle, 42% of parents recognized that children will be smokers if they lived with smoking parents, and a higher percentage (64%) was reported by Hanoi parents, \( p=0.042 \). Approximately 70% of Newcastle parents agreed that “Children who are exposed to ETS are more likely to develop asthma” compared to about 90% of parents of asthma in Hanoi, \( p=0.007 \). Parents in Newcastle and Hanoi both agreed that children were likely to have asthma attacks if they were exposed to ETS (78.72% versus 84.4%, \( p=0.16 \)).
How to prevent ETS exposure in children with asthma

Table 3.4.5: Attitudes of smoking parents of children with asthma to prevent ETS exposure in children

<table>
<thead>
<tr>
<th>Attitudes of smoking parents ( % subjects)</th>
<th>Smoking parents in Newcastle ( n=9)</th>
<th>Smoking parents in Hanoi ( n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wanting to quit smoking</td>
<td>77.8</td>
<td>70</td>
</tr>
<tr>
<td>Ever trying to quit smoking</td>
<td>66.7</td>
<td>80</td>
</tr>
<tr>
<td>Only smoking in house after children have gone to bed</td>
<td>33.3</td>
<td>10</td>
</tr>
<tr>
<td>Only smoking in a separate closed door room with open window for ventilation</td>
<td>22.2</td>
<td>70</td>
</tr>
<tr>
<td>Only smoking outside</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>Never smoking in car when children are present</td>
<td>66.7</td>
<td>90</td>
</tr>
<tr>
<td>Considering to quit smoking</td>
<td>77.8</td>
<td>90</td>
</tr>
<tr>
<td>Establishing a dry, warm and comfortable outdoor smoking area.</td>
<td>55.6</td>
<td>50</td>
</tr>
<tr>
<td>Taking to GP about smoking and children’s health</td>
<td>55.6</td>
<td>100</td>
</tr>
<tr>
<td>Using Nicotine replacement therapy</td>
<td>55.6</td>
<td>70</td>
</tr>
<tr>
<td>Connecting to the Quit line and requesting to call back counselling</td>
<td>44.4</td>
<td>80</td>
</tr>
<tr>
<td>Trying until success</td>
<td>44.4</td>
<td>80</td>
</tr>
</tbody>
</table>
The knowledge and attitudes of smoking parents of children with asthma to prevent the harmful effects of ETS exposure on their children’s health are described in Table 3.4.5. Of smoking parents, 78% in Newcastle and 70% in Hanoi wanted to quit smoking. Also, 66.7% of smoking parents in Newcastle and 80% of smoking parents in Hanoi had previously tried to quit smoking.

In the group of parental smoker in Newcastle, all believed that only smoking outside may prevent the harmful effects of smoking in children. Never smoking in the car when children are present and/or considering quitting smoking were likely to be an option to prevent ETS exposure of Newcastle smoking parents. However, only 44.4% of smoking parents of children with asthma thought that they should keep trying until successful.

In the group of parental smokers of children with asthma in Hanoi, 70% or more of smoking parents thought that only smoking in a separate closed door room with a window open for ventilation, or only smoking outside, or never smoking in a car when children are present, or considering to quit smoking, or taking to GP about smoking and children’s health, or using Nicotine replacement therapy, or connecting to the Quit line and requesting to call back counselling were helpful to prevent ETS exposure in their children. Also 80% of them expected that they would keep trying until successful.

However, the number of smoking parents of children with asthma in each group was small, and this may not reflect the real knowledge and attitudes of smoking parents towards passive smoking.
Discussion

We investigated the knowledge and attitudes of parents of children with asthma in the two cities: Newcastle (Australia) and Hanoi (Vietnam). One person in each family was interviewed and most responders were females (97.5% in Newcastle and 71.8% in Vietnam). The prevalence of children with asthma living with smoking parents in Hanoi was higher than that in Newcastle. However, the prevalence of ETS exposure was higher in children with asthma in Newcastle compared to children with asthma in Hanoi. This may reflect different ETS exposure pattern in the two cities.

Smoking is a substantial problem not only in developing countries but also in developed countries. Vietnam has one of the highest prevalence of smokers in the world. A 2004 survey in Hanoi, Ho Chi Minh City, and 2 rural areas in Vietnam of men and women aged 18 years or older found that the smoking prevalence among men was 72.8% and 4.3% among women (615). This explains the high prevalence of children with asthma in Hanoi who lived with smokers (about 50%). However, no correlation was found between ETS exposure in children with asthma and parental smoking in Hanoi. Interestingly, there was a significant correlation between smoking parents and ETS exposure in children with asthma in Newcastle. The difference in ETS exposure in children between the two cities may be related to which parent smoked. In this study, mothers were often the chief carer of children, and the predominant participant in each city. Maternal smoking is more often associated with children’s exposure to ETS than others who smoked in their households, especially at high levels of tobacco use (555, 573). This study found that not only was there a higher prevalence of parental smoking in Hanoi compared to Newcastle, but all of the smokers in Hanoi were males. No mothers in Hanoi reported
themselves to be smokers and this may result in fewer effects of ETS exposure in children. On the other hand, while the prevalence of parents of childhood asthma who smoked in Newcastle was lower, 7.6% of children with asthma lived with maternal smoking and 9.1% of them had both a mother and father who smoked. This pattern of smoking may have stronger effects on ETS exposure in children with asthma. In addition, more than 50% of parents of children with asthma worked part time, compared to more than 80% of parents in Hanoi who worked full time. This suggests that Newcastle mothers may have more direct contact time with their children than mother in Hanoi. If the mothers from Hanoi were smokers; then their children would be at higher risk of ETS exposure. These findings may partly explain why the prevalence of ETS exposure in children with asthma in Hanoi was lower than children with asthma in Newcastle. In addition, all children in Hanoi had low level ETS exposure whereas 14.6% of children with asthma in Newcastle had high ETS exposure.

The influence of passive smoking on the health of children has been emphasized in many studies, especially the relationship between ETS exposure and childhood diseases such as respiratory illness, cancers and sudden infant death syndrome (250, 549). Parents of children with asthma in the two cities both recognized that ETS exposure may cause dangerous diseases in children such as asthma, emphysema, heart diseases, lung cancer, smaller babies and SIDS. Whether or not the respondent was a smoker, most of them agreed that passive smoking caused asthma in children. This is an interesting finding, given that the parents were aware of their child’s asthma diagnosis.
More than 30 carcinogenic chemicals are present in tobacco smoke (616). Studies report that passive smoking causes similar adverse health effects to active smoking, especially in young children. Exposure to passive smoking increases the risk of cancer such as lung cancer (617), breast cancer (616), bowel cancer (618), brain tumor (619), prostate cancer (620), and skin cancer (621). Passive smoking also causes or contributes to respiratory diseases such as asthma (95, 319, 323, 622), emphysema (623, 624), ear infection in children (625), tonsillitis in children (626, 627), SIDS (628) and vascular diseases such as stroke (629), and heart attacks (630). Prenatal smoking or mothers who were exposed to ETS during pregnancy is associated with low birth weight babies being born (631, 632) or lower levels of intelligence in their children (633, 634). Whether passive smoking may contribute to increase risks of other diseases is still inconsistent, but studies have reported relationships between ETS exposure and blindness (635), cataracts (635), deafness (636), arthritis (637), erection problems (638), meningococcal diseases in children (639), eczema (593, 640), and fainting (641). ETS exposure may impair the children’s development and increase risks of severe diseases.

The parents of children with asthma in the two cities showed different attitudes towards passive smoking. Parents of children with asthma in both cities recognized the potential hazards of ETS exposure for children, with equal or more than 90% of parents agreeing with the statements that “Children should have the right to live in a smoke-free home” and “An act should be passed which forbids all indoor smoking in the vicinity of children”. However, while only 3% of parents of children with asthma in Hanoi agreed that “Adults have a right to smoke wherever they want in their own homes”, 23% of
parents of children with asthma in Newcastle agreed with this statement. The different attitudes of the parents of the two cities may be explained by the smoking habits of responders. Most of the responders were females. Although the prevalence of parental smoking in Hanoi was higher than that in Newcastle, most of the responders were females who were non-smokers that resulted in the negative attitude towards smoking. In contrast, approximately 17% of mothers in Newcastle reported themselves to be smokers, which may lead them having a less negative attitude towards smoking.

Parental smoking may recruit children to become smokers (411, 642). A significant relationship between smoking parents and their children smoking may be one of the major health hazards of ETS exposure by parents. A survey in the Nordic countries found that about 50% of adult smokers and 25% of non-smokers were not aware of the role of smoking parents in recruiting smokers (408). Another survey in 9,008 young adolescents (aged 11-16 years) from the Netherlands reported that children with current asthma were at high risk of being a current regular smoker, and the risk increased if parents smoked. In contrast, anti-smoking policies and parental smoking abstinence resulted in lower odds of children smoking. However, this study also found that despite the hazards of smoking, the parents of adolescents with asthma were more likely to smoke than parents of children without asthma (557).

Many studies have identified the significant relationship between parental smoking and the development of asthma in children (95, 323, 643). Epidemiologic studies report that children who live in homes with cigarette smokers have a higher risk of developing
asthma than children who stayed in homes of non smokers (324). Asthma incidence is
increased in children who live with smoking mothers (325, 326). The odds ratio for
being diagnosed with asthma in children who were exposed to ETS was 1.78 compared
to those not exposed to ETS (95% CI: 1.33-2.31) (328). A significantly higher proportion
of parents of children with asthma in Hanoi agreed that ETS exposure can induce asthma
development in children in comparison with parents of children with asthma in
Newcastle, suggesting a difference in smoking type of responders as well as a difference
in the culture between the two cities. It suggests a need for specific education on the
harmful effects of ETS on asthma.

An association between passive smoking and increased asthma exacerbations among
children is reported (250, 337). The parents of children with asthma in Newcastle and the
parents of children with asthma in Hanoi both recognized the link between ETS exposure
and asthma attacks in children.

This study found that parents of children with asthma in the two cities were aware of the
harmful effects of ETS exposure in their children and more than 70% of smokers wanted
to quit smoking. Although the prevalence of children with asthma who lived with
smokers was still high in the two cities, more than 50% of smokers had low levels of
nicotine addiction. This suggests an important opportunity for behavioral strategies to
assist in smoking cessation. Interestingly, most of smokers thought that smoking only
outside may prevent ETS exposure in children. However, we found that despite smokers
only smoking outside, 75% of children had been exposed to ETS (Chapter III). The
findings suggest that behaviour such as smoking in the house after children have gone to
bed, smoking in a separate room or smoking only outside may not prevent ETS exposure in children. Smoking cessation is a unique method to improve the smokers and their children’s health. Unfortunately, smoking cessation is difficult and requires more attempts from smokers. Understanding the dangerous effects of smoking may help smokers to quit smoking. All children have a right to stay in a free smoking environment.

Conclusion

The prevalence of parental smoking of children with asthma is still high in Newcastle and Hanoi. Parents of children with asthma, especially smoking parents should be more aware of the harmful effects of smoking on their children health and themselves. Smoking parents may also recruit their children to be smokers.

Smoking cessation is to be encouraged for all smokers. The health risk awareness helps parental smoking alter their smoking behavior as well as protecting children from ETS exposure. There is an opportunity to increase awareness of the specific harmful effects of smoking on asthma.
PART IV

SUMMARY AND CONCLUSION

Asthma is a chronic respiratory disease that is particularly common in Australia. Over 2.2 million Australians are currently diagnosed with asthma (156). The prevalence of asthma in children is also high, accounting for 14-16% of children (one in six) (156). Despite the progress in the management of childhood asthma, the prevalence of the disease seems to be increasing.

Asthma is defined as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular, eosinophils, mast cells, neutrophils, T lymphocytes, macrophages, and epithelial cells (439). Airway inflammation is suggested to cause the recurrent episodes of asthma, which are characterized by asthma symptoms such as wheezing, breathlessness, and coughing, particularly at night or in the early morning. Inflammation also causes an increase in bronchial responsiveness to a variety of stimuli (439). Airway inflammation is a key factor of the disease and investigation of airway inflammation is a treatment target in asthma, especially in children.

Airway inflammation can be assessed through both invasive and non invasive methods. In children, non invasive methods are safe and feasible for investigating airway inflammation. Non-invasive methods such as exhaled nitric oxide, exhaled breath condensate and sputum induction have been used to assess airway inflammatory markers in both healthy children and children with asthma. However, there have been few studies
to compare these markers to each other in the assessment of airway inflammation in childhood asthma.

**Sampling methods**

Non-invasive sampling methods have different success rates. This study found that EBC collection was successful in nearly 100% of children, that more than 80% of children could perform FeNO measurements, and that adequate sputum samples were obtained in only 64% of children. The correlations between airway inflammatory markers measured by different methods and subject characteristics, atopy and AHR were different for each method. EBC pH was not associated with any subject characteristics, atopy or AHR, and did not discriminate healthy children from those with asthma. FeNO levels were significantly associated with demographic characteristics such as age, height, weight and BMI. Also, an increase in FeNO levels reflected the atopic status and AHR in children with asthma. Induced sputum sample collection provided a picture of cells and mediators involved in airway inflammation. Sputum eosinophils may reflect the airway obstruction in children with asthma, and positive correlations were found between sputum eosinophils, atopic status and AHR.

A comparison of airway inflammatory markers between the different sampling methods found that EBC pH was not associated with either FeNO levels or sputum eosinophils, but there was a positive correlation between FeNO levels and sputum eosinophils.
Clinical characteristics of children with asthma were different compared to healthy children. EBC pH failed to detect and difference between healthy children and children with asthma. FeNO levels and sputum eosinophils were significantly higher in children with asthma than healthy children. In contrast, sputum macrophages were higher in healthy children than children with asthma. The findings suggest that FeNO measurements and induced sputum are useful methods to assess airway inflammation in children with asthma. FeNO has the advantage of being easy to perform and with a higher success rate. EBC is an efficient sampling method, but markers other than EBC pH need to be identified and evaluated for use in order to investigate airway inflammation.

Inflammatory phenotypes

Asthma has typically been considered as a disease that was related to atopy (644-646) and eosinophilic bronchitis (206, 647). There is now increasing evidence that other cells such as neutrophils, macrophages, and epithelial cells play an important role in the inflammatory processes in asthma. Adult asthma is now considered as a heterogeneous chronic inflammation of airways (172, 189) where many cells take part (5, 172). The role of non-eosinophilic asthma, especially in children is not fully understood. Investigating the pattern of airway inflammation in childhood asthma may provide useful information for prognosis and treatment. This thesis reports a study of asthma inflammatory phenotypes in children.

The subtypes of asthma were categorized based on induced sputum eosinophil and neutrophil counts. The study sought to identify stable phenotypes, where children
displayed the same phenotype over at least a 3 month period. In contrast to studies in adults, there were two main phenotypes of airway inflammation identified in children with asthma: **Eosinophilic asthma (EA)** with % sputum eosinophils more than 2.5% and % sputum neutrophils less than 61%, and **Paucigranulocytic asthma (PGA)** with % sputum eosinophils were equal or less than 2.5% and % sputum neutrophils less than 61%. These 2 subtypes had different clinical features. Children with PGA had less severe disease compared to children with increased sputum eosinophils. This was demonstrated by less asthma symptoms in the last 12 months, lower asthma control score, and a lower maintenance ICS dose than children with increased sputum eosinophils. In contrast, children with EA showed increased airway obstruction and AHR compared to children with PGA. The proportion of persistent asthma in the PGA group was significantly lower compared to the eosinophilic group.

In summary, there were two dominant phenotypes of airway inflammation in children with asthma: the more severe of the two, eosinophilic asthma, had worse airway obstruction and increased AHR compared to PGA. These children had increased sputum eosinophils and FeNO levels, but lesser sputum macrophages. Comparatively, children with PGA had less severe symptoms, no increase in eosinophils, a normal FeNO and increased sputum macrophages.

This study reported that increased sputum eosinophils were associated with asthma severity. Previous publications also demonstrate that eosinophilic airway inflammation may reflect the current clinical state such as wheezing during the past 12 months (647),
frequency of nocturnal symptoms (518), poorly controlled asthma (522), \( \beta_2 \)-agonist requirements (518), persistent asthma symptoms (491, 508, 521), a higher risk of asthma attacks in the 12 months (522) amongst other clinical variables of asthma (178, 179, 523).

This study found that asthma without increased sputum eosinophils was common in childhood asthma. Interestingly, the sputum cells of children with a PGA pattern were similar to healthy children, and there was no difference in FeNO levels between healthy children and children with PGA. These findings explain why clinical features of asthma were less severe in children with PGA compared to children with EA. Neutrophilic asthma was uncommon, possibly because the features associated with a persistent sputum neutrophilia are uncommon in children.

Induced sputum can categorize the pattern of airway inflammation, however the success rate is less than other non-invasive methods and execution of the technique is more difficult, especially in children. With the remarkable differences in both clinical symptoms and the objective measurements between EA and PGA, I suggest that a combination of non-invasive markers such as FeNO measurement in combination with atopy, lung function, or AHR may predict the pattern of airway inflammation in children with asthma. This study found that high FeNO levels combined with low \( \text{FEV}_1/\text{FVC} \) ratio gave the highest predictive value to detect eosinophilic asthma. The findings may provide a useful way to investigate the pathogenic mechanism and treatment responses in
childhood asthma. The sample size of this study was small and further research with a greater number of children is needed to validate these results.

*Environmental tobacco smoke exposure*

The mechanisms that establish different airway patterns in asthma need further investigation. While allergy has been seen as the main mechanism in atopic asthma, the mechanisms leading to the development of non-atopic or non-eosinophilic asthma are not fully understood. Some studies suggest that non-eosinophilic asthma is associated with environmental factors. Another possible explanation of PGA is that the clinical symptoms are driven by macrophage activation, or other mechanisms such as mast cell infiltration in airway smooth muscle (201) or neurogenic mechanisms.

The relationship between parental smoking and the development of asthma in children has been mentioned in many studies (95, 323). Passive smoking exposure is associated with increased asthma symptoms (133), and a persistent deficit in lung function of children (334). In adults, active smoking induces a neutrophilic response (348).

In this study, different methods to assess ETS exposure in children were compared. This included parental reports, exhaled CO measurement and children’s urinary cotinine measurement. Cotinine is one of major metabolites of nicotine. It reflects the degree of tobacco smoke exposure (250), and can be useful to assess ETS exposure in non-smokers due to the long half life of cotinine.
This study found that ETS exposure was high among children with asthma. Children who lived with smoking parents had a significantly higher risk of exposure to ETS compared to children living with non-smoking parents, especially if the parents smoked indoors. The longitudinal design of the study allowed an assessment at asthma during periods with and without ETS exposure.

Several deleterious effects of ETS exposure on airway pathophysiology in children with asthma were reported in this study. Lung function was reduced during ETS exposure. ETS exposure was associated with changing airway inflammation compared to a non-ETS exposed period, including reduced FeNO, lower EBC pH, increased sputum neutrophils and decreased sputum eosinophils. The mechanisms of reduced sputum eosinophils and elevated sputum neutrophils with ETS exposure may be similar to that in smokers with asthma. Toxic substances derived from cigarette smoke can contribute to attenuate airway eosinophil influx (350) or lead to an increase in eosinophil apoptosis (349), resulting in decreased sputum eosinophils. The pattern of airway inflammation in children with asthma who were exposed to ETS was not stable over time; and it depended on the time and the levels of ETS exposure. Currently, we do not know whether airway inflammation returns to baseline after ETS exposure or whether there is a difference in airway inflammation in children with asthma having short term or long term ETS exposure.

The level of airway neutrophilia seen with ETS exposure was less than that reported in adults. However, there may be an age related effect on sputum neutrophils with normal
sputum neutrophils being less in children than adults. A study by Simpson et al in adults reported that the median of % sputum neutrophils was approximately 25% in healthy adults and asthma was classified as Neutrophilic if % sputum neutrophils were more than 61% (161). The normal baseline level for % sputum neutrophils in healthy children has not been reported. The median of % sputum neutrophils in healthy children in this study was 12%. The % of sputum neutrophils of children with asthma during an ETS exposed period was higher (26.5%). Further studies with larger sample sizes are needed to investigate the cut point to differentiate between normal and higher levels of sputum neutrophil counts in children. The data in this study suggest that if children have sputum neutrophils more than 25%, then this may represent increased sputum neutrophils.

Sputum macrophages may play an important role in airway inflammation in children with PGA. This study found that sputum macrophages were similar between healthy children and children with PGA (more than 80%). However, sputum macrophages were only 56% in children with EA. When children with asthma were exposed to ETS, % sputum eosinophils were significantly decreased, but % sputum macrophages were unchanged (69%), and lower than healthy children. The findings suggest that although children with PGA and children with asthma who were exposed to ETS had been classified as non-eosinophilic asthma, the pattern of airway inflammation and clinical symptom were not similar. The role of macrophages in airway inflammation in children with asthma is currently not clear and more studies are needed to assess this. Investigation and classification of airway phenotypes in children with asthma using sputum eosinophils and neutrophils may be not enough to differentiate the different subtypes of airway
inflammation in children with asthma. With the evidence that many cells play a role in airway inflammation in children with asthma, the classification of airway phenotypes may need to consider the role of other cells, such as macrophages, in addition to sputum eosinophils and neutrophils.

Beside the interesting findings, this study also had some limitations. Currently, the number of studies assessing airway inflammation in children with asthma is limited. The question of whether airway inflammation in childhood asthma was similar to adult asthma was not fully addressed in prior studies. While this study has categorized two phenotypes of airway inflammation in children with asthma, the factors that may contribute to modify airway pattern when children grow up are less defined. Although we believe it is unlikely that children with PGA were children with EA who were taking ICS, it is undeniable that ICS may reduce sputum eosinophils. Previous studies have reported that non-eosinophilic asthma in adults was associated with severe asthma, but this study demonstrated that children with PGA was less severe compared to children with EA. The controversial findings may explain by there were more than one group in asthma with non- eosinophilic pattern. It also highlights another important difference in airway inflammation childhood asthma compared to adult asthma.

This study had observed that non-eosinophilic asthma was main characteristic of children who were exposed to ETS. However, the airway pattern at the ETS exposure period was temporary. We did not know when airway inflammation returns to the baseline. The long
term effects of ETS exposure in children with asthma were suggested to modify airway inflammatory pattern, but the mechanisms of changing the airway pattern are unknown.

In order to further understand the airway inflammatory pattern in children with asthma, a study with a greater number of children is needed to validate these results. Investigation of the pattern of AI in both stable and exacerbation periods in the same subjects with asthma will be conducted to assess the relationship between asthma triggers and pattern of AI. While the role of airway eosinophils was mentioned in previous studies, the role of other cells, especially neutrophils and macrophages in children with asthma should be more investigated. I hypothesize that the ratio of airway neutrophils and macrophages may predict the severity of asthma.

ETS exposure was harmful for both healthy children and children with asthma. A study with a long-term follow-up would be useful to assess airway modification in children with a high risk of ETS exposure, such as children living with parental smoking. Non-eosinophilic asthma was the main characteristic of children who were exposed to ETS. Little data is available on the impact of passive smoking on asthma treatment. However it is known that airway inflammation in non-eosinophilic asthma has responded poorly to ICS. A randomized controlled trial in childhood asthma is needed to assess the corticosteroid resistance in children who are exposed to ETS.

In conclusion, the studies in this thesis describe the relative value of different non-invasive sampling methods to assess airway inflammation in childhood asthma.
Important differences from adult asthma are the existence of two main phenotypes (not four) and the ability to use clinical and laboratory markers to distinguish these subtypes. In addition, a deteriorated biological effect on airway inflammation has been demonstrated to occur following ETS exposure, with the induction of non-eosinophilic mechanisms. These findings contribute to the understanding of airway inflammation in childhood asthma and suggest areas for future research studies to assess the clinical relevance of these findings.
REFERENCES


96. Lodrup Carlsen K, Carlsen K. Effects of maternal and early tobacco exposure on the development of asthma and airway hyperreactivity.[see comment]. [Review] [41 refs]. Current Opinion in Allergy & Clinical Immunology 2001;1(2):139-43.
126. Rosas I, McCartney H, Payne R. Analysis of the relationships between enviromental factors (aero-allergens, air pollution, and whether) and asthma emergency admissions to a hospital in Mexico City. Allergy 1998;53:394-401.


578. Mitchell E, Stewart A. The ecological relationship of tobacco smoking to the prevalence of symptoms of asthma and other atopic diseases in children: the International
APPENDICES

Table of Appendices

APPENDIX I: Information sheet and consent form
I a- Information sheet for parents of healthy children
I b- Information sheet for healthy control young people 12 to 17 years of age
I c- Information sheet for healthy control children 7 to 11 years of age
I d- Information sheet for parents of children with asthma
I e- Information sheet for young people with asthma 12 to 17 years of age
I f - Information sheet for children with asthma 7 to 11 years of age
I g- Consent form

APPENDIX II: Asthma symptom questionnaire

APPENDIX III: Asthma control questionnaire

APPENDIX IV: Passive smoking exposure questionnaire

APPENDIX V: Knowledge and attitude of parents towards passive smoking

APPENDIX VI: Study data record form
VI a- Exhaled NO worksheet
VI b- Exhaled CO worksheet
VI c- Allergy skin prick test worksheet

VI d- Saline challenge and sputum induction worksheet

APPENDIX VII: Sputum and EBC processing worksheet
APPENDIX I: INFORMATION SHEET AND CONSENT FORM

I a - INFORMATION SHEET FOR PARENTS OF HEALTHY CONTROL CHILDREN

Title: Airway inflammation in stable childhood asthma

Investigators: Professor Peter Gibson
               Professor Michael Hensley
               Doctor Bruce Whitehead
               Doctor Nguyen Thi Dieu Thuy

Purpose of the study
We would like to invite you and your child to participate in a study being undertaken as part of the requirements for a PhD at the University of Newcastle by Dr Nguyen Thi Dieu Thuy, under the supervision of Professor Peter Gibson and Professor Michael Hensley.

Asthma is one of the most common chronic diseases in children. Airway inflammation is an important feature of asthma and the target of preventer treatment. The type of inflammation may determine the benefits of treatment. We have recently found that some people with asthma have a different type of inflammation, called non eosinophilic asthma. We do not know if it occurs in children, whether it is stable over time, or how it is triggered.

The purpose of this study is to investigate this pattern of airway inflammation in childhood asthma. We need to compare the markers of inflammation including cells and chemicals found in the airways of children with asthma to those found in healthy children to determine any differences.

You and your child should be aware that your child is being invited to participate as a healthy control and your child's participation in the study will further our understanding
of asthma and its management, but it may not necessarily benefit you or your child personally

**Who can participate?**

We wish to recruit children aged between 7 to 17 years. Children will be recruited from the John Hunter Children's Hospital and from the community through responses to the media.

**Inclusion Criteria**

- Children between 7 and 17 years of age

**Exclusion Criteria**

- Use of any asthma medication
- Diagnosis of any respiratory disease
- Diagnosis of respiratory infection within the preceding 4 weeks
- Treatment with antibiotics during the preceding 4 weeks
- Children with other significant morbidity (other illness)

If your child has any of the exclusion criteria he/she will not be eligible to participate. However once your child has completed antibiotics or after a respiratory tract infection, he/she may be eligible to participate.

**What procedures will be carried out?**

If you and your child agree to take part in the study, you will be invited to attend for 1 to 2 visits to the Respiratory Department at the John Hunter Hospital. Each visit lasts no longer than 2 hours. A second visit may be necessary to obtain sufficient sputum to perform all investigations. This visit would be within 2 weeks of the initial visit.

The following procedures will be performed at each visit:

1. We will ask you and your child a series of questions about your child’s general health, quality of life and passive smoking exposure. These will take about 20 minutes.
We will also ask you some questions about your attitudes to smoking. This takes about 15 minutes.

2. Your child will be asked to undergo an induced sputum test using an inhaled salt solution. This test is safe and involves your child breathing in a salty air mixture. The mixture can cause your child to cough, but this is quickly resolved by taking reliever medicine, which will be provided. Your child might need to perform this test at both visits for the researchers to collect enough sputum.

We will monitor all your child's procedures. If your child's lung function drops by more than 15% from his/her baseline during the sputum test, we will give him/her the reliever medicine. We will check your child's lung function again to ensure that your child's lung function is safe and the sputum procedures will be continued. If your child's lung function drops by more than 15% from his/her baseline a second time, we will stop sputum test and administer further reliever medicine and monitor your child until his/her lung function returns to normal.

3. Your child will also be asked to perform other breathing tests, which are safe and do not hurt.

4. Your child will undergo a skin allergy test. This involves placing a drop of allergy extract on the forearm, scratching the surface, and measuring the response 15 minutes later. The allergens tested will be fungi (aspergillus fumigatus), dust mite, cockroach, cat hair, dog hair and grass mix.

5. We will collect some saliva from your child. Your child will be asked to spit saliva into the sterilized container. We will collect it to measure a chemical, which relates to environmental tobacco smoke exposure.

6. We will also collect urine from your child. Some chemicals in urine will be measured in order to detect whether or not your child is exposed to a smoking environment.
You and your child can stop any of the tests at any time.

We will stop the tests if you or your child feels uncomfortable.

For children aged 14 or younger, a parent/carer will need to be present with the child for all the testing and questions. If your child is older than 14 years of age, you may wish not to be present. This is up to you and your child.

**Risk/Benefits**

**Risks**
The sputum test can cause coughing, some discomfort in your child's chest and wheezing. This is brief and responds promptly to reliever medication (Salbutamol), which will be readily available.
There are no known side effects to having the other breathing tests.
The skin test may cause the skin to become temporarily itchy if your child is allergic to these substances. This may only last for an hour and your child will be given some cream to relieve the itch.

**Benefits**
Your child's participation in this study will benefit our understanding of asthma but you or your child may not benefit personally from the study. We also ask your permission to contact your child’s general practitioner to inform them that your child is participating in the study. The results of breathing tests and the skin prick test can be sent to your child's general practitioner at your request. At the completion of the study, we can also provide the study results to you. However, you should be aware that this study could take several years to complete.

**Is the information confidential?**
Only your child's doctor and the study staff will know that your child is taking part in this study. All information relating to this study will be kept confidential and your child will
not be identifiable in any reports of the study. Participants will be identified for the purposes of the study by initials and an assigned trial number.

All data collected for the purposes of the study will be transcribed into a separate folder in addition to the medical records, and participants will not be identifiable from these folders. Only authorised staff working on the study will have access to the data and information collected during the study. All information relating to the study will be kept securely for 15 years following the completion of the study.

**Voluntary participation**

Your child's participation in this study is voluntary. You or your child may decide to withdraw from the study at any time. Study information and samples collected prior to this will be retained as they will still be useful for the purposes of the study. The data must also be retained for regulatory reporting if there is an adverse event. If the study ceases prematurely all data collected up to that point would be retained for analysis. The study doctor may take your child out of the study if it is deemed that your child is unable to follow the study protocol or they become ineligible for the study.

Thank you for your time.

Prof P Gibson  
Senior Staff Specialist.  

Prof M Hensley  
Director, Department of Respiratory and Sleep Medicine

Dr B Whitehead  
Senior Staff Specialist

Dr Nguyen Thi Dieu Thuy  
PhD student
Complaints

This project has been approved by the University’s Human Research Ethics Committee, Approval No: H-877-0804 and the Hunter Area Research Ethics Committee of Hunter New England Health, Reference:04/04/07/3.09.

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to

The Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, telephone (02 49216333, email Human-Ethics@newcastle.edu.au or

Dr Nicole Gerrand, Professional Officer, Hunter Area Research Ethics Committee, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305, telephone (02) 49214950, email Nicole.Gerrand@hnehealth.nsw.gov.au.
I b- INFORMATION SHEET FOR HEALTHY CONTROL YOUNG PEOPLE
12 TO 17 YEARS OF AGE.

Title: Airway inflammation in stable childhood asthma

Investigators:  
Professor Peter Gibson  
Professor Michael Hensley  
Doctor Bruce Whitehead  
Doctor Nguyen Thi Dieu Thuy

Purpose of the study
We would like to invite you to participate in a study being undertaken as part of the requirements for a PhD at the University of Newcastle by Nguyen Thi Dieu Thuy, under the supervision of Professor Peter Gibson and Professor Michael Hensley.

Asthma is one of the most common chronic diseases in children. Airway inflammation is an important feature of asthma and the target of preventer treatment. The type of inflammation may determine the benefits of treatment. We have recently found that some people with asthma have a different type of inflammation, called non eosinophilic asthma. We do not know if it occurs in children, whether it is stable over time, and how it is triggered. The purpose of this study is to investigate this pattern of airway inflammation in childhood asthma. We need to compare the markers of inflammation including cells and chemicals found in the airways of children with asthma to those found in healthy children to determine any differences.

You should be aware that you are being invited to participate as a healthy control and your participation in the study will further our understanding of asthma and its management, but it may not necessarily benefit you personally.

Who can participate?
We will recruit children aged between 7 to 17 years. Children will be recruited through the John Hunter Children's Hospital and from the community through media.
Inclusion Criteria

- Children between 7 to 17 years of age

Exclusion Criteria

- Use of any asthma medication
- Diagnosis of any respiratory disease
- Diagnosis of respiratory infection within the preceding 4 weeks
- Treatment with antibiotics during preceding 4 weeks
- Children with other significant morbidity (other illness)

If you have any of the exclusion criteria you will not be eligible to participate. However, once you have ceased antibiotics or when your respiratory tract infection has resolved you may participate.

What procedures will be carried out?

If you agree to take part in the study, you will be invited to attend for one or two visits to the Respiratory Department at the John Hunter Hospital. Each visit lasts no longer than 2 hours. A second visit may be necessary to obtain sufficient sputum to perform all investigations. This visit would be within 2 weeks of the initial visit.

The following procedures will be performed at each visit:

1. We will ask you a series of questions about your general health, quality of life and passive smoking exposure, these will take about 20 minutes.

2. You will be asked to undergo an induced sputum test using an inhaled salt solution. This test is safe and involves you breathing in a salty air mixture. The mixture can cause you to cough, but this is quickly resolved by taking reliever medicine, which will be provided. You might need to do this test at both visits for the researchers to collect enough sputum.
We will monitor all your procedures. If your lung function drops by more than 15% from your baseline when you perform the sputum test, we will give you the reliever medicine. We will check your lung function again to ensure that your lung function is safe and the sputum procedures will be continued. If your lung function drops by more than 15% from your baseline a second time, we will stop sputum test and administer further reliever medicine and monitor you until your lung function returns to normal.

3. You will also be asked to perform other breathing tests, which are safe and do not hurt.

4. You will undergo a skin allergy test. This involves placing a drop of allergy extract on the forearm, scratching the surface, and measuring the response 15 minutes later. 5. The allergens tested will be fungi (aspergillus fumigatus), dust mite, cockroach, cat hair, dog hair and grass mix.

5. We will collect some saliva from you. You will be asked to spit saliva into the sterilized container. We will collect it to measure a chemical, which relates to environmental tobacco smoke exposure.

6. We will also collect some urine from you. Some chemicals in urine will be measured in order to detect whether or not you are exposed to a smoking environment.

You can stop any of the tests at any time.

We will stop the tests if you feel uncomfortable.

If you would like, you may have your mum and/or dad with you during all procedures.

Risk/Benefits

Risks
The sputum test can cause coughing, some discomfort in your chest and wheezing. This is brief and responds promptly to reliever medication (Salbutamol), which will be readily available.

There are no known side effects to having the other breathing tests.

The skin test may cause the skin to become temporarily itchy if you are allergic to these substances. This may only last for an hour and you will be given some cream to relieve the itch.

Benefits

Your participation in this study will benefit our understanding of asthma but you may not benefit personally from the study. We also ask your permission to contact your general practitioner to inform them that you are participating in the study. The results of breathing tests and the skin prick test can be sent to your general practitioner at your request. At the completion of the study, we can also provide the study results to you. However, you should be aware that this study could take several years to complete.

Is the information confidential?

Only your doctor and the study staff will know that you are taking part in this study. All information relating to this study will be kept confidential and you will not be identifiable in any reports of the study. Participants will be identified for the purposes of study by initials and an assigned trial number.

All data collected for the purposes of the study will be transcribed into a separate folder in addition to the medical records, and participants will not be identifiable from these folders. Only authorised staff working on the study will have access to the data and information collected during the study. All information relating to the study will be kept securely for 15 years following the completion of the study.

Voluntary participation

Your participation in this study is voluntary. You may decide to withdraw from the study at any time, however study information and samples collected prior to this will still be retained as they are useful for the purposes of the study. The data must also be retained
for regulatory reporting if there is an adverse event. If the study ceases prematurely all data collected up to that point would be retained for analysis. The study doctor may take you out of the study if it is deemed that you are unable to follow the study protocol or become ineligible for the study.

Thank you for your time,

Prof P. Gibson
Senior Staff Specialist

Prof M Hensley
Director, Department of Respiratory and Sleep Medicine

Dr B Whitehead
Senior Staff Specialist

Dr Nguyen Thi Dieu Thuy
PhD student

Complaints
This project has been approved by the University’s Human Research Ethics Committee, Approval No: H- 877-0804 and the Hunter Area Research Ethics Committee of Hunter New England Health, Reference: 04/04/07/3.09.

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, telephone (02 49216333, email Human-Ethics@newcastle.edu.au or, to Dr Nicole Gerrand, Professional Officer, Hunter Area Research Ethics Committee, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305, telephone (02) 49214950, email Nicole.Gerrand@hnehealth.nsw.gov.au
I c- INFORMATION SHEET FOR HEALTHY CONTROL CHILDREN

7 TO 11 YEARS OF AGE

Title: Airway inflammation in stable childhood asthma

Investigators: Professor Peter Gibson
Professor Michael Hensley
Doctor Bruce Whitehead
Doctor Nguyen Thi Dieu Thuy.

My name is Doctor Thuy, I am a student from the University of Newcastle and working with Professor Peter Gibson and Professor Michael Hensley. We would like to invite you to participate in my research project named "Airway inflammation in stable childhood asthma".

A lot of children get asthma and part of this is may be because of something called airway inflammation. To understand it further, we need to compare the cells in the airways of children with asthma to those of healthy children.

We are asking you to allow us to collect some of your spit. You will be asked to inhale salty air from a machine. This may make you cough a little. We will collect whatever you are able to cough up. Doing this test can cause your airways to tighten up but we can give you medicine to stop this.

We will also ask you to perform some other breathing tests and ask you and your mum or dad some questions. These tests do not hurt; your mum or dad will be with you at all times.

You will be asked to undergo a skin allergy test. This test may cause your skin to become itchy but we will give you some cream to stop the itch.

If you want to stop any of the tests at any time, you can.
You may be asked to attend up to two visits at the John Hunter Hospital. Each visit lasts no longer than two hours.

If you are upset by anything that happens in this study, you should talk to your parents and they will know whom to contact.

Thank you for your interest.

Prof Peter Gibson
Senior Staff Specialist

Prof Michael Hensley
Director, Department of Respiratory and Sleep Medicine.

Dr Bruce Whitehead
Senior Staff Specialist

Dr Thuy Nguyen.
PhD student
I d-INFORMATION SHEET FOR PARENTS OF CHILDREN WITH ASTHMA

Title: Airway inflammation in stable childhood asthma

Investigators: Professor Peter Gibson
               Professor Michael Hensley
               Doctor Bruce Whitehead
               Doctor Nguyen Thi Dieu Thuy

Purpose of the study
We would like to invite you and your child to participate in a study being undertaken as part of the requirements for a PhD at the University of Newcastle by Dr Nguyen Thi Dieu Thuy, under the supervision of Professor Peter Gibson and Professor Michael Hensley. Your child's participation in the study will further our understanding of asthma and its management, but it may not necessarily benefit you or your child personally.

Asthma is one of the most common chronic diseases in children. Airway inflammation is an important feature of asthma and the target of preventer treatment. The type of inflammation may determine the benefits of treatment. We have recently found that some people with asthma have a different type of inflammation, called non eosinophilic asthma. We do not know if it occurs in children, whether it is stable over time, or how it is triggered. The purpose of this study is to investigate this pattern of airway inflammation in childhood asthma.

Who can participate?
We will recruit children aged between 7 to 17 years with doctor diagnosed asthma. Children with asthma will be recruited from the John Hunter Children's Hospital and from the community through media.

Inclusion Criteria
- Children between 7 to 17 years of age
- A doctor’s diagnosis of stable asthma
Exclusion Criteria

- Increase in symptoms including wheeze, shortness of breath or cough or features of a lower respiratory tract infection
- Increase in asthma medication use
- Oral steroid use
- Visit to the GP or hospital due to worsening asthma

If your child has any of these exclusion criteria within the preceding 4 weeks, they will not be eligible to participate. However after your child’s asthma has returned to normal they may participate.

*What procedures will be carried out?*

If you and your child agree to take part in the study, you and your child will be invited to attend between one and three visits to the Respiratory Department at the John Hunter Hospital. Each visit lasts no longer than 2 hours. Visit 2 and Visit 3 will be three months and six months after the initial visit.

If you and your child agree to participate in the study, your child needs to withhold their morning dose of reliever and controller medicine. These include:

- Ventolin
- Respolin
- Airomir
- Asmol
- Serevent
- Seretide
- Oxis
- Foradile
- Symbicort
If your child wishes to use their reliever medicine for symptom relief for exercise this will be permitted. We will ask you at the study visit what medications your child takes and when they received their last dose.

The following procedures will be performed at each visit:

1. We will ask you and your child a series of questions about your child’s asthma symptoms, quality of life and passive smoking exposure; these will take about 20 minutes.

We will also ask you some questions about your attitudes to smoking. This takes about 15 minutes.

2. Your child will be asked to undergo a sputum test using a salt solution. This test is safe and involves your child breathing in a salty air mixture. The mixture can cause your child to cough, but this is quickly recovered by taking reliever medicine, which will be provided.

We will monitor your child’s asthma. If your child’s lung function drops by more than 15% from his/her baseline during the sputum test, we will give him/her the reliever medicine. We will check your child’s lung function again to ensure that your child’s lung function is safe and the sputum procedures will be continued. If your child’s lung function drops by more than 15% from his/her baseline a second time, we will stop the sputum test and administer further reliever medicine and monitor your child until his/her lung function returns to normal.

3. Your child will also be asked to perform other breathing tests. All techniques are safe and do not hurt.

4. Your child will undergo a skin allergy test. This involves placing a drop of allergy extract on the forearm, scratching the surface, and measuring the response 15 minutes later. The allergens tested will be fungi (aspergillus fumigatus), dust mite, cockroach, cat hair, dog hair and grass mix.
5. We will collect some saliva from your child. Your child will be asked to spit saliva into the sterilized container. We will collect it to measure a chemical level, which relates to environmental tobacco smoke exposure.

6. We will also collect urine from your child. Some chemicals in urine will be measured in order to detect whether or not your child is exposed to a smoking environment.

7. We will ask you and your child’s permission to be contacted by phone every 2 weeks over a 1 year period after visit 1. We will ask you or your child (if he/she is more than 14 years of age) some questions about worsening asthma.

You and your child can stop any of the tests at any of the time.
We will stop the tests if your child feels uncomfortable or his/her asthma becomes worse. For children aged 14 or younger, a parent/carer will need to be present with the child for all the testing and questions. If your child is older than 14 years of age, you may wish not to be present. This is up to you and your child.

**Risk/Benefits**

*Risks*
The sputum test can cause coughing, some discomfort in your child’s chest and wheezing. This is brief and responds promptly to reliever medication (Salbutamol), which is readily available.
There are no known side effects to having the other breathing tests.
The skin test may cause the skin to become temporarily itchy if your child is allergic to these substances. This may only last for an hour and your child will be given some cream to relieve the itch.

*Benefits*
Your child’s participation in this study will benefit our understanding of asthma but you or your child may not benefit personally from the study. We also ask your permission to contact your child’s general practitioner to inform them that your child is participating in
the study. The results of breathing tests and the skin prick test can be sent to your child’s general practitioner at your request. At the completion of the study, we can also provide the study results to you. However, you should be aware that this study could take several years to complete.

**Is the information confidential?**

Only your child’s doctor and the study staff will know that your child is taking part in this study. All information relating to this study will be kept confidential and your child will not be identifiable in any reports of the study. Participants will be identified for the purposes of study by initials and an assigned trial number. All data collected for the purposes of the study will be transcribed into a separate folder in addition to the medical records, and participants will not be identifiable from these folders. Only authorized staff working on the study will have access to the data and information collected during the study. All information relating to the study will be kept securely for 15 years following the completion of the study.

**Voluntary participation**

Your child’s participation in this study is voluntary. You or your child may decide to withdraw from the study at any time. Study information and samples collected prior to this will be retained as they will still be useful for the purposes of the study. The data must also be retained for regulatory reporting if there is an adverse event. If the study ceases prematurely all data collected up to that point would be retained for analysis. The study doctor may take your child out of the study if it is deemed that your child is unable to follow the study protocol or they become ineligible for the study.

Thank you for your time.

Prof P Gibson
Senior Staff Specialist

Prof M Hensley
Director, Department of Respiratory and Sleep Medicine
Dr B Whitehead  
Senior Staff Specialist

Dr Nguyen Thi Dieu Thuy  
PhD student

Complaints

This project has been approved by the University’s Human Research Ethics Committee, Approval No: H-877-0804 and the Hunter Area Research Ethics Committee of Hunter New England Health, Reference:04/04/07/3.09.

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, telephone (02) 49216333, email Human-Ethics@newcastle.edu.au or to Dr Nicole Gerrand, Professional Officer, Hunter Area Research Ethics Committee, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305, telephone (02) 49214950, email Nicole.Gerrand@hnehealth.nsw.gov.au.
Title: Airway inflammation in stable childhood asthma

Investigators: Professor Peter Gibson
Professor Michael Hensley
Doctor Bruce Whitehead
Doctor Nguyen Thi Dieu Thuy

Purpose of the study
We would like to invite you to participate in a study being undertaken as part of the requirements for a PhD at the University of Newcastle by Dr Nguyen Thi Dieu Thuy, under the supervision of Professor Peter Gibson and Professor Michael Hensley.

Your participation in the study will further our understanding of asthma and its management, but it may not necessarily benefit you personally.

Asthma is one of the most common chronic diseases in children. Airway inflammation is an important feature of asthma and the target of preventer treatment. The type of inflammation may determine the benefits of treatment. We have recently found that some people with asthma have a different type of inflammation, called non eosinophilic asthma. We do not know if it occurs in children, whether it is stable over time, or how it is triggered. The purpose of this study is to investigate this pattern of airway inflammation in childhood asthma.

Who can participate?
We will recruit children and aged between 7 to 17 years with doctor diagnosed asthma. Children with asthma will be recruited from the John Hunter Children's Hospital and from the community through media.

Inclusion Criteria
- Children between 7 to 17 years of age
- A doctor’s diagnosis of stable asthma
Exclusion Criteria

- Increase in symptoms including wheeze, shortness of breath or cough or features of a lower respiratory tract infection
- Increase in asthma medication use
- Oral steroid use
- Visit to the GP or hospital due to worsening asthma

If you have any of these exclusion criteria within the preceding 4 weeks, you will not be eligible to participate. However when your asthma has returned to normal you may participate.

What procedures will be carried out?

If you agree to take part in the study, you will be invited to attend between one and three visits to the Respiratory Department at John Hunter Hospital. Each visit lasts no longer than 2 hours. Visit 2 and Visit 3 will be three months and six months after the initial visit.

If you agree to participate in the study, you will need to withhold your morning dose of reliever and controller medicine these include any of the following:
- Ventolin
- Respolin
- Airomir
- Asmol
- Serevent
- Seretide
- Oxis
- Foradile
- Symbicort
If you wish to use your reliever medicine for symptom relief for exercise this will be permitted. We will ask you at the study visit what medications you take and when you took your last dose.

The following procedures will be performed at each visit:
1. We will ask you a series of questions about your asthma symptoms, quality of life and passive smoking exposure, these will take about 20 minutes.

2. You will be asked to undergo a sputum induction test using an inhaled salt solution. This test is safe and involves you breathing in a salty air mixture. The mixture can cause you to cough, but this is quickly resolved by taking reliever medicine, which will be provided.
   We will monitor your asthma. If your lung function drops by more than 15% from your baseline when you perform the sputum test, we will give you the reliever medicine. We will check your lung function again to ensure that your lung function is safe and the sputum procedures will be continued. If your lung function drops by more than 15% from your baseline a second time, we will stop sputum test and administer further reliever medicine and monitor you until your lung function returns to normal.

3. You will also be asked to perform other breathing tests, which are safe and do not hurt.

4. You will undergo a skin allergy test. This involves placing a drop of allergy extract on the forearm, scratching the surface, and measuring the response 15 minutes later. The allergens tested will be fungi (aspergillus fumigatus), dust mite, cockroach, cat hair, dog hair and grass mix.

5. We will collect some saliva from you. You will be asked to spit saliva into the sterilized container. We will collect it to measure a chemical, which relates to environmental tobacco smoke exposure.
6. We will also collect urine from you. Some chemicals in urine will be measured in order to detect whether or not you are exposed to a smoking environment.

7. We ask your permission to be contacted by phone every 2 weeks over a 1 year period after visit 1. We will ask you some questions about worsening asthma.

You can stop any of the tests at any time.

We will stop the tests if you feel uncomfortable or your asthma becomes worse.

If you would like, you may have your mum and/or dad with you for all procedures.

We also ask your and your parent permission to be contacted by phone each 2 weeks until 3 months after the last visit to ask some questions about asthma exacerbations.

**Risk/Benefits**

*Risks*

The sputum test can cause coughing, some discomfort in your chest and wheezing. This is brief and responds promptly to reliever medication (Salbutamol), which is readily available.

There are no known side effects to having the other breathing tests.

The skin test may cause the skin to become temporarily itchy if you are allergic to these substances. This may only last for an hour and you will be given some cream to relieve the itch.

*Benefits*

Your participation in this study will benefit our understanding of asthma but you may not benefit personally from the study. We also ask your permission to contact your general practitioner to inform them that you are participating in the study. The results of breathing tests and the skin prick test can be sent to your general practitioner at your request.
completion of the study, we can also provide the study results to you. However, you should be aware that this study could take several years to complete.

**Is the information confidential?**

Only your doctor and the study staff will know that you are taking part in this study. All information relating to this study will be kept confidential and you will not be identifiable in any reports of the study. Participants will be identified for the purposes of study by initials and an assigned trial number.

All data collected for the purposes of the study will be transcribed into a separate folder in addition to the medical records, and participants will not be identifiable from these folders. Only authorised staff working on the study will have access to the data and information collected during the study. All information relating to the study will be kept securely for 15 years following the completion of the study.

**Voluntary participation**

Your participation in this study is voluntary. You may decide to withdraw from the study at any time, however study information and samples collected prior to this will still be retained as they are useful for the purposes of the study. The data must also be retained for regulatory reporting if there is an adverse event. If the study ceases prematurely all data collected up to that point would be retained for analysis. The study doctor may take you out of the study if it is deemed that you are unable to follow the study protocol or become ineligible for the study.

Thank you for your time

Prof P Gibson
Senior Staff Specialist

Prof M Hensley
Director, Department of Respiratory and Sleep Medicine.
Complaints

This project has been approved by the University’s Human Research Ethics Committee, Approval No: H-877-0804 and the Hunter Area Research Ethics Committee of Hunter New England Health, Reference: 04/04/07/3.09. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, telephone (02) 49216333, email Human-Ethics@newcastle.edu.au or, to Dr Nicole Gerrand, Professional Officer, Hunter Area Research Ethics Committee, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305, telephone (02) 49214950, email Nicole.Gerrand@hnehealth.nsw.gov.au.
Title: Airway inflammation in stable childhood asthma

Investigators:  
Professor Peter Gibson  
Professor Michael Hensley  
Doctor Bruce Whitehead  
Doctor Nguyen Thi Dieu Thuy.

My name is Doctor Thuy, I am a student from the University of Newcastle and working with Professor Peter Gibson and Professor Michael Hensley. I would like to invite you to participate in my research project named "Airway inflammation in stable childhood asthma".

A lot of children get asthma and part of this may be because of something called airway inflammation. Airway inflammation is important in asthma and we do not fully understand it.

If you agree to participate in the study, we would like you not to have your morning asthma medication, we will explain this to your mum or dad.

We are asking you to allow us to collect some of your spit. You will be asked to breath in some salty air from a machine. This may make you cough a little. We will collect whatever you are able to cough up. Doing this test can cause your airways to tighten up but we can give you medicine to stop this.

We will also ask you to perform some other breathing tests and ask you and your mum or dad some questions. These tests do not hurt, your mum or dad will be with you at all times.

You will be asked to undergo a skin allergy test. This test may cause your skin to become itchy but we will give you some cream to stop the itch.
If you want to stop any of the tests at any time, you can.
You may attend up to three visits at the John Hunter Hospital. Each visit lasts for two hours.

If you are upset by anything that happens in this study, you should talk to your parents and they will know who to contact.

Thank you for your time.

Prof Peter Gibson
Senior Staff Specialist

Prof Michael Hensley
Director, Department of Respiratory and Sleep Medicine

Dr Bruce Whitehead
Senior Staff Specialist

Dr Thuy Nguyen
PhD student
I g - CONSENT FORM

Study Title: *Airway inflammation in stable childhood asthma.*

*Investigators:*

Prof Peter Gibson  
Prof Michael Hensley  
Dr Bruce Whitehead  
Dr Nguyen Thi Dieu Thuy

**Parent and Child Consent to participate in research.**

I have read the study information provided to me and understand all points.

*I/My child………………………………agree/s to participate in the above research project and give my consent freely.*

I understand that the project will be conducted as described in the Information sheet, a copy of which I have retained.

*I understand I/ my child can withdraw from the project at any time and do not have to give any reason for withdrawing, but that any samples collected prior to this will be retained.*

*I consent to complete the questionnaires and I agree for my child to undergo the breath and sputum tests.*

*I understand that my child's personal information will remain confidential to the researchers.*

*I have had the opportunity to have questions answered to my satisfaction.*

Name of Parent/ Guardian: …………………………………….
Signature of Parent/Guardian:…………………………………
Signature of Child (optional): ……………………………………
Date  :……………………………………
APPENDIX II: ASTHMA SYMPTOM QUESTIONNAIRE

SUBJECT DEMOGRAPHICS

SUBJECT NUMBER: .......................... MRN:....................

SURNAME ................................. FIRST NAME..................

ADDRESS..............................................................................

SUBURB.................................. POST CODE......................

PHONE HOME.............................. WORK.........................

MOBILE.................................E-MAIL.............................

SEX: MALE/FEMALE( CIRCLE) DATE OF BIRTH.........................

HEIGHT......................................WEIGHT......................
INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA

- Children between 7 to 17 years of age
- A doctor’s diagnosis of stable asthma

EXCLUSION CRITERIA

In the preceding 4 weeks

- Increasing the asthma symptoms including wheeze, shortness of breath or cough or features of a lower respiratory tract infection.
- Increasing the use of reliever medication (Ventolin, Bricanyl) or using a nebuliser.
- Needing a course of oral steroid (Prednisolone)
- Reducing activities because of asthma.
- Attending your doctor or hospital because of asthma.

Note to researcher: If the answer to any of these questions in the exclusion criteria is yes, the subject cannot continue with the study today and needs to be rescheduled for another day when stable.
CLINICAL DATA

ASTHMA SYMPTOMS

1- How old was your child when diagnosed with asthma?
   a- 1 year old
   b- 1 year to 2 years.
   c- 2 years to 3 years.
   d- 3 years to 4 years.
   e- >4 years.

   If (e), age: years:

Wheezing History

2- Has your child ever wheezed? Yes/No

   (If no, go to question 5)

   If yes: At what age did wheezing begin?
   a- 1 year old.
   b- 1 year to 2 years.
   c- 2 years to 3 years.
   d- 3 years to 4 years.
   e- >4 years.

   If (e), age: years:

3- When did your child last wheeze?
   a- < 1 week.
   b- 1 week - 1 month.
   c- 1-6 months.
   d- 6-12 months.
   e- > 12 months.
4- In the past year, how often did your child wheeze?
   a- daily
   b- < daily but > 2 times per week
   c- monthly
   d- less than monthly
   f- no wheeze

5- Has your child ever wheezed during exercise? Yes/No

**General History**

6- In the past 12 months, did your child ever experience episodes of breathlessness? Yes/No

   If yes: How often are these episodes?
   a- daily
   b- <daily but more than 2 times per week.
   c- 1-2 times per week.
   d- monthly
   e- < no breathless episodes.

7- Is your child more breathless at night? Yes/No

8- In the past 12 months, has your child ever had a dry cough at night? Yes/No.

   If yes: How often does this occur?
   a- nightly
   b- >2 episodes, < 7 episodes per week.
   c- 1-2 per week
   d- monthly
   e- <monthly
   f- no dry cough.
9- In the past 12 months, has your child been woken by his/her asthma symptoms?
   Yes/No

   If yes: How often?
   a- nightly
   b- > 2 but <7 nights per week
   c- 1-2 nights per week
   d- monthly
   e- < monthly
   f- never woken up.

10- In the past 12 months, how many chest colds has your child had?
   a- 0
   b- 1
   c- 2-3
   d- 4 or more.

11- When your child was a baby did anyone living in your house smoke?
    Yes/No

    If yes: Relationship to child

   Family history

12- Has the child's natural mother ever had:
    a- Asthma
    b- Hay fever
    c- Eczema
    d- Allergies
    e- Chest disease (apart from asthma)

13- Does your child have any siblings?
   Yes/No

   If yes: How many?
14- Do any of the siblings have asthma/eczema/allergic rhinitis?  Yes/No.
    If yes, which disease:

_Hay Fever questions_

These next questions are about problems which occur when your child DOES NOT have
a cold or the flu.

15- Has your child ever had a problem with sneezing, or a runny, or blocked nose when
he/she did not have a cold or the flu?        Yes/No
    _If no- skip to question 22_

16- In the past 12 months, has your child had a problem with sneezing, or a runny, or
blocked nose when he/she did not have a cold or the flu?        Yes/No.

17- During the past 12 months, has this nose problem been accompanied by itchy
    -watery eyes ?        Yes/No

18- In which of the past 12 months did this nose problem occur? (circle which apply)
    
    January                     July
    February                   August
    March                      September
    April                      October
    May                        November
    June                       December

19- In the past 12 months, how much did your child's nose symptoms interfere with daily
    activity?
    a-  Not at all
    b-  A little
    c-  A moderate amount
    d-  A lot
20- Has your child ever had hay fever? Yes/No

*Asthma History*

21- In the past 12 months, how many asthma attacks has your child had?
   a- No attacks
   b- 1 attack
   c- 2-3 attacks
   d- 4-12 attacks
   e- >12 attacks.

22- When was your child's last asthma attack?
   a- < 1 week
   b- 1 week-1 month
   c- 1 to 6 months
   d- 6 to 12 months
   e-> 12 months.

23- Has your child ever been hospitalized for an asthma attack (exacerbation)? Yes/No
   If yes: Age of child yrs.

24- In the past 12 months has your child been hospitalized for an asthma attack (exacerbation) Yes/No

25- Approximately how long do these attacks last for? (days)

26- In the past 12 months has your child presented to the emergency department due to an asthma attack? Yes/No
   If yes: How many times?
27- Has your child visited your local doctor for an asthma attack in the past 12 months?
   If yes: How many times?
     a- 0
     b- 1
     c- 2-3
     d- 4 or more

28- How many of these periods have last longer than 7 days
   Nil                           <5                        5-10                    >10

29- In the past 12 months, how many days has your child lost from school due to their asthma?
   a- 0
   b- 1
   c- 2-3
   d- 4 or more

Medication History

30- Has your child ever taken medication for their asthma? Yes/No
   If yes: When did they first begin taking medication
     a- < 12 months ago.
     b- >12 months ago

31- In the past 12 months, how often has your child needed to take their medications?
   a- never
   b- daily
   c- >once a month
   d- <once a month
32- Does your child use inhaled beta-agonists?  Yes/No
   If yes: How often?
   a- daily: How many times during day  times
   b- weekly
   c- monthly
   d- <monthly
   e- does not use

33- Has your child ever been prescribed a course of oral Prednisone?  Yes/No
34- Current medications

<table>
<thead>
<tr>
<th>Please circle</th>
<th>Current</th>
<th>Last 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short acting $\beta_2$- agonist</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>(Ventolin, Asmol, Airomir, Bricanyl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long acting $\beta_2$- agonist</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>(Serevent, Oxis, Foradite)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cromoglycate (Intal, Tilade)</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Leukotriene modifier (Singularair)</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Short acting anticholinergic (Atrovent, Atrovent Forte)</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Long acting anticholinergic (Spiriva)</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Nasal steroids</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Theophylline (Neulin)</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Oral Steroids (Prednisolon)</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Type:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reducing Dose?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhaled Corticosteroids (Qvar, Pulmicort, Flixotide)</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose (strength, puffs, frequency)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eg: Fluticasone 250mcg, 2puffs bd=250x2x2=1000mcg TDD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seretide/Symbicort (circle one)</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Dose (strength, puffs, frequency)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Medications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Asthma triggers:

<table>
<thead>
<tr>
<th>TRIGGERS</th>
<th>YES</th>
<th>NO</th>
<th>DON’T KNOW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral Infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergy Exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise Induced</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarette Smoke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atmospheric Pollutants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weather Change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emotional Stress</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Associated Conditions**

### Does your child have?

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastro-oesophageal Reflux</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic Rhinitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX III: ASTHMA CONTROL QUESTIONNAIRE

1- On average, during the past week, how often were you woken by your asthma during the night?
   0- Never
   1- Hardly ever
   2- A few minutes
   3- several times
   4- Many times
   5- A great many times
   6- Unable to sleep because of asthma

2- On average, during the past week, how bad were your asthma symptoms when you woke up in the morning?
   0- No symptoms
   1- Very mild symptoms
   2- Mild symptoms
   3- Moderate symptoms
   4- Quite severe symptoms
   5- Severe symptoms
   6- Very symptoms

3- In general, during the past week, how limited were you in your activities because of asthma?
   0- Not limited at all
   1- Very slightly limited
   2- Slightly limited
   3- Moderately limited
   4- Very limited
   5- Extremely limited
   6- Totally limited
4- In general, during the past week, how much **shortness of breath** did you experience because of your asthma?
   0- None
   1- A very little
   2- A little
   3- A moderate amount
   4- Quite a lot
   5- A great deal
   6- A very deal

5- In general, during the past week, how much of the time did you **wheeze**?
   0- Not at all
   1- Hardly any of the time
   2- A little of time
   3- A moderate amount of the time
   4- A lot of the time
   5- Most of the time
   6- All the time

6- On average, during the past week, how many **puffs of short-acting bronchodilator** (eg. Ventolin) have you used each day?
   0- None
   1- 1-2 puffs most days
   2- 3-4 puffs most days
   3- 5-8 puffs most days
   4- 9-12 puffs most days
   5- 13-16 puffs most days
   6- More than 16 puffs most days
7- FEV\textsubscript{1} pre- bronchodilator…………………………………… 0 - > 95% predicted
  FEV\textsubscript{1} predicted…………………………………………… 1 - 95-90%
  FEV\textsubscript{1} % predicted……………………………………… 2- 89-80%
  (Record actual values on the dotted lines
  and score the FEV\textsubscript{1} % predicted in the next column) 3- 79- 70%
  4- 69-60%
  5- 59-50%
  6- < 50% predicted

AVERAGE SCORE: 7/7
APPENDIX IV: PASSIVE SMOKING EXPOSURE QUESTIONNAIRE

Relationship of person interview to the child……………………………………
( e.g., Mother, Father, Uncle, Foster Mother).

Who is the child’s chief carer ……………………………………………………
( e.g., Mother, Father, Uncle, Foster Mother).

Occupation:………………………………………………………………………

1- Do you allow smoking in your house? (tick the appropriate result).
   a- No one is allowed to smoke in my home. [ ]
   b- Smoking is allowed in the home but not in the same room as my child. [ ]
   c- No one who lives in the home is allowed to smoke but exception is made for
      visitors. [ ]
   d- No restrictions on smoking at home. [ ]
   e- Other (please specify)…………………………………………………

*If the answer to question 1 was a, omit questions 2, 3, 4, 5 and ask question 6.*

2- Does your child live in the same household with someone who smoke? YES/NO
   If yes

3- How many people in your household smoke?

   Relationship:

   Cigarettes/day: /day.
Relationship:

Cigarettes/day /day.

4- How many times per week do visitors smoke INSIDE your home?

<1 1 2 3 4 5 6 7 >7

Number of cigarettes smoked per visit (circle approximately)

<1 1-5 6-10 11-15 16-20 >20

5- How many times per week do visitors smoke OUTSIDE your home?

<1 1 2 3 4 5 6 7 >7

Number of cigarettes smoked per visit (circle approximately)

<1 1-5 6-10 11-15 16-20 >20

6- How much time does your child spend in homes/public buildings/cars where other people smoke?

a- Does not visit smoky places. [ ]

b- Hours per week INDOORS 1-5 6-10 11-15 16-20 >20

c- Hours per week OUTDOORS 1-5 6-10 11-15 16-20 >20

*If the answer to question 6 was b or c, ask question 7*
7-How much smoke do you think your child is exposed to in other homes/buildings/cars etc?

   a-Dense smoke
   b-Moderate smoky
   c-Slightly smoky

*Please collect a urine sample on all participants and measure urinary cotinine by Nic Alert Test.*
APPENDIX V: KNOWLEDGE AND ATTITUDE OF PARENTS TOWARDS PASSIVE SMOKING

SUBJECT NUMBER: DATE: INITIALS:

AGE……………………………………………………………………..

GENDER: Male Female

MARITAL STATUS: Single Married Divorced Other

ETHNICITY:……………………………………………………………………

EDUCATION: Primary school High school TAFE University

EMPLOYMENT STATUS: Full-time Part-time Unemployed Student
Section 1

1- Have you ever smoked any cigarettes, cigars or pipes? YES NO

If the answer to question 1 was NO, omit all questions in the sections 1, 2, and 4 and ask all questions in the section 3.

If YES

1.1 - Have you smoked more than 100 cigarettes, cigars or pipes since the pregnancy of your first child? YES/NO

1.2- Have you smoked any cigarettes, cigars or pipes in the last 4 weeks? YES/NO

1.3- How many cigarettes, cigars or pipes do you smoke on an average day?

1.4- How many cigarettes, cigars or pipes do you smoke INSIDE your house on an average day?

0 1 2 3 4 5 6 7 8 9 10 ..................30

1.5-Is your child/children ever present in the same room, in which you are smoking? YES/NO

If YES, how often:

- Never
- Occasionally
- Often
- Every day

1.6 -How many cigarettes, cigars or pipes do you smoke OUTSIDE your house on an average day?
1.7- On an average day, how many cigarettes, cigars or pipes do you smoke when you are driving in a car?

1.8- Is your child/children ever present in the car when you are smoking?

   YES/NO

   If YES, how often:

   - Never
   - Occasionally
   - Often
   - Every day

1.9- How many hours per week do you think your child is exposed to ETS?

   0  30min  1h  2  3  4  5  6  7  >7
**Section 2**

| Points |  
|--------|---|
| **2.1-** How soon after you wake up do you smoke your first cigarette? |  
| Within 5 minutes | 3 |
| 6-30 min | 2 |

|  
| **2.2-** Do you find it difficult to refrain from smoking in places where it is forbidden, e.g. in church, at the library, in cinema etc? |  
| Yes | 1 |
| No | 0 |

|  
| **2.3-** Which cigarette would you hate most to give up? |  
| The first one in the morning | 1 |
| All other | 0 |

|  
| **2.4-** How many cigarette/day do you smoke? |  
| 10 or less | 0 |
| 11-20 | 1 |
| 21-30 | 2 |
| 31 or more | 3 |

|  
| **2.5-** Do you smoke more frequently during the first hours after waking than during the rest of the day? |  
| Yes | 1 |
| No | 0 |

|  
| **2.6-** Do you smoke if you are so ill that you are in the bed most of the day? |  
| Yes | 1 |
| No | 0 |
Section 3

Can you answer TRUE or FALSE?

3.1-The second hand smoke from cigarettes is known to cause:

<table>
<thead>
<tr>
<th>Condition</th>
<th>TRUE</th>
<th>FALSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bowel Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain Tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erection problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart attacks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma attacks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emphysema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blindness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deafness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cataracts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smaller babies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower levels of intelligence in children.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear infections in children.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tonsillitis in children.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meningococcal diseases in children.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eczema in children.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fainting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIDS (sudden infant death syndrome)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Please circle one in each question

3.2-Adults have a right to smoke wherever they want in their own homes.
   a- Agreed completely
   b- Tended to agree
   c- Tended to disagree
   d- Disagreed completely

3.3- Children should have the right to live in a smoke-free home.
   a- Agreed completely
   b- Tended to agree
   c- Tended to disagree
   d- Disagreed completely

3.4- An act should be passed which forbids all indoor smoking in the vicinity of children.
   a- Agreed completely
   b- Tended to agree
   c- Tended to disagree
   d- Disagreed completely

3.5- Children who are exposed to environmental tobacco smoke are more likely to start to smoke themselves.
   a- No, not at all.
   b- Maybe/maybe not
   c- Probably yes
   d- Definitely yes
3.6- Children who are exposed to ETS are more likely to develop asthma.
   a- No, not at all.
   b- Maybe/maybe not
   c- Probably yes
   d- Definitely yes

3.7- Children who are exposed to ETS are more likely to have asthma attacks.
   a- No, not at all.
   b- Maybe/maybe not
   c- Probably yes
   d- Definitely yes

Section 4
4.1 - Do you want to quit smoking? YES/NO
4.2 - Have you ever tried to quit smoking? YES/NO
4.3 - What would you be prepared to do to prevent harm to your child from ETS?

*Please circle one in each question*
4.3.1 - Only smoke in the house after children have gone to bed.
   a- Definitely NOT
   b- Probably NOT
   c- Unsure
   d- Perhaps YES
   e- Definitely YES

4.3.2 - Only smoke in a separate closed door room with open window for ventilation.
   a- Definitely NOT
   b- Probably NOT
   c- Unsure
   d- Perhaps YES
   e- Definitely YES
4.3.3- Only smoke outside
   a - Definitely NOT
   b- Probably NOT
   c - Unsure
   d- Perhaps YES
   e - Definitely YES

4.3.4- Never smoke in the car when children are present.
   a- Definitely NOT
   b- Probably NOT
   c- Unsure
   d- Perhaps YES
   e- Definitely YES

4.3.5- Consider quitting smoking.
   a- Definitely NOT
   b- Probably NOT
   c- Unsure
   d- Perhaps YES
   e- Definitely YES

4.3.6- Establish a dry, warm and comfortable outdoor smoking area.
   a- Definitely NOT
   b- Probably NOT
   c- Unsure
   d- Perhaps YES
   e- Definitely YES
4.3.7- Talk to my GP about smoking and the children’s health.
   a- Definitely NOT
   b- Probably NOT
   c- Unsure
   d- Perhaps YES
   e- Definitely YES

4.3.8- Get and use Nicotine Replacement Therapy (NRT) from my pharmacist.
   a- Definitely NOT
   b- Probably NOT
   c- Unsure
   d- Perhaps YES
   e- Definitely YES

4.3.9- Connect to the Quit line and request call back counseling.
   a- Definitely NOT
   b- Probably NOT
   c- Unsure
   d- Perhaps YES
   e- Definitely YES

4.3.10- Keep trying to get support and help to quit smoking until I succeed
   a- Definitely NOT
   b- Probably NOT
   c- Unsure
   d- Perhaps YES
   e- Definitely YES
APPENDIX VI: STUDY DATA RECORD FORM

VI a- NITRIC OXIDE MEASUREMENT

NO TESTING

SUBJECT NUMBER: ……………..DATE…………..INITIALS: …………..

TIME SINCE LAST MEAL:
(If the child has been eating or drinking less than 2 hours before performing test, please note what food and/or drink consumed)

TIME OF MEASUREMENT:

MODE USED:          CLINICAL/ RESEARCH

EXHALED NO READING:
1. 
2. 
3. 
AVERAGE EXHALED NO (ppb):

AMBIENT NO (ppb):

COMMENTS

CHECKLIST:
1. Demonstrate how to perform in research mode.
2. Return to clinic mode and record 3 readings.
3. If unable to get a reading in clinic mode return to research mode and record 3 readings.
VI b- EXHALED CARBON MONOXIDE LEVEL

Subject No: ……………………………… Date ……………………….Initials: ………….  

1- Does your child currently smoke?                    YES                     NO 

2- Has your child ever smoked ?                         YES            NO( NEVER SMOKER)  
( > 100 cigarettes = YES) 

Exhaled Carbon Monoxide Level:              ppm (Smokalyzer)
VI c- ALLERGY SKIN PRICK TEST

Subject Number:……………………Date……………Initials……………

Antihistamine withheld?            Yes                    No

<table>
<thead>
<tr>
<th>Test</th>
<th>Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td></td>
</tr>
<tr>
<td><strong>Allergens</strong></td>
<td></td>
</tr>
<tr>
<td><em>Alternaria Tenius</em></td>
<td></td>
</tr>
<tr>
<td>Dust mite</td>
<td></td>
</tr>
<tr>
<td>Cockroach mix</td>
<td></td>
</tr>
<tr>
<td>Grass mix</td>
<td></td>
</tr>
</tbody>
</table>

Atopy positive:               Yes                       No
( Any allergen ≥ 3 mm)
**VI d- SALINE CHALLENGE AND SPUTUM INDUCTION**

**Were medications withheld?** Yes/No

<table>
<thead>
<tr>
<th>Nebuliser Make</th>
<th>Spirometer Make</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted FEV₁/FVC</td>
<td>FEV₁/FVC (best of 3 attempts)</td>
</tr>
<tr>
<td>Nebuliser cup pre test weight</td>
<td>Nebuliser cup post test weight</td>
</tr>
</tbody>
</table>

Asthma Medications and time last taken

<table>
<thead>
<tr>
<th>Saline Nebulisation Time</th>
<th>FEV₁</th>
<th>% fall from baseline FEV₁</th>
<th>Sputum Produced</th>
<th>Ventolin given (dose &amp; time given)</th>
<th>FEV₁ post Ventolin</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td>Spontaneous Y/N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 sec</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

60
VII- SPUTUM AND EBC PROCESSING WORKSHEET

AICA (Airway inflammation in childhood asthma)

Sample Data Sheet.

<table>
<thead>
<tr>
<th>Initials ________</th>
<th>Study No. ________</th>
<th>Sputum Type _______</th>
<th>Visit No. _______</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slide No __________</td>
<td>EBC No ___________</td>
<td>Date <strong><strong>/</strong></strong>/____</td>
<td></td>
</tr>
</tbody>
</table>

**Macroscopic Description**

- **Volume**: ________ mL
- **Colour**: ________
- **Colour No.**: ________
- **Plugs**: ________
- **Other**: ________

**Sample Processing**

- **Sputum Used**: ________ µL
- **MGG**: (1) C2R: (1)
- **PLP ICC**: (2) NE ICC: (2)
- **Spare**: (6)
- **Supernatant**: (6)
- **Ultracentrifuge**: (2)
- **PCR sample (100µL/600µL RLT)**: (2)
- **Cell suspension stored**: Y / N

**EBC**

- **Volume**: ________ µL
- **Supernatant**: ________ (x 220µL, )
- **Deaerated**: Y / N
- **pH after deaeration**: ________

**Alive____ Dead____**

**Viability ________%**

- **TCC** = (1 + 2) x 0.02
  
  = ________ X 10⁶ / mL

**Vol** = _____ x mLs

  = _____ mL

**Cytospin Quality**

- **Debris**: 4 (nil), 3 (scant), 2 (moderate), 1 (excessive) ______
- **Cell Outline**: 4 (preserved), 3 (isolated cell damage), 2 (many cells damaged)
  1 (most cells damaged) ______
- **Nuclear Morphology**: 4 (preserved), 3 (isolated nuclei damage), 2 (many nuclei damage)
  1 (most nuclei damaged) ______
- **Squamous**: 4 (<20%), 3 (21–60%), 2 (61–85%), 1 (>85%) ______
- **Overall Impression**: 4 (good), 3 (acceptable), 2 (just acceptable), 1 (bad) ______
- **Slide macrophages present**: 1 = yes 0 = no ______
- **Number of cells on slide**: 0 = <200 1 = 200-399 2 ≥ 400 ______

**Total Quality /23**

- **Macrophage Inclusions**: absent 1+ 2+ 3+
- **Free Granules**: absent 1+ 2+ 3+
<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col. Epithelial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils (C2R)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Visit 1 photo taken?  Y / N

Process by

| Date | / | /

Counted by

| Date | / | /