Lung Function in Healthy South African Adult Females

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PREAMBLE
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List of Abbreviations

ACCP    American College of Chest Physicians

ANZSRS  Australian and New Zealand Society of Respiratory Science

APSR    Asian Pacific Society of Respirology

ATS     American Thoracic Society

BMI     Body Mass Index

COPD    Chronic Obstructive Pulmonary Disease

CRF     Case Report Form

DALY    Disability Adjusted Life Years

DCHS    Drakenstein Child Health Study

ERS     European Respiratory Society

FEF25-75% Forced Midexpiratory Flow Rate

FEV\textsubscript{1} Forced Expiratory Volume in 1 Second

FVC     Forced Vital Capacity

GINA    Global Initiative for Asthma

GLI     Global Lung Initiative

GOLD    Global Initiative for Chronic Obstructive Lung Disease

HIV     Human Immunodeficiency Virus
LLN  Lower Limit of Normal
LMIC  Lower and Middle Income Countries
MMEF  Maximal Mid-Expiratory Flow
NCD  Non-Communicable Disease
NHANES  National Health and Nutrition Examination Survey
PEF  Peak Expiratory Flow
SES  Socio-Economic Status
TB  Tuberculosis
TSANZ  Thoracic Society of Australia and New Zealand
ULN  Upper Limit of Normal
WHO  World Health Organisation
WHS  World Health Survey
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Contents

List of Tables 8

A: PROTOCOL 10

1. Introduction 11
2. Methods 16
3. Time frame 20
4. Dissemination of research findings 21

Appendix A1: Post-hoc power calculation 22

B: LITERATURE REVIEW 25

1. Introduction and objectives of this literature review 26
2. Search Strategy 26
3. The Burden of Adult Lung Disease in Low to Middle Income Countries 26
4. Common Respiratory Diseases in South Africa 30
5. Spirometry 35
6. The Role of Reference Equations in Spirometry 36
7. The Global Lung Initiative 37

References 42

Appendix B1: GLI Reference Equations 45
List of Tables

Figure A1: Volume Time Curve
Table A1: Timeframe for study schedule
Table B1: Spirometric Classification of COPD Severity
Table B2: Comparison of Asthma and COPD
Table B3: Percentage difference in mean pulmonary function by sex and ethnic group compared to Caucasians
Table B4: Example parameters for the fields L, mu and sigma used in the GLI reference equations
Table C1: Anthropometric, demographic and socioeconomic characteristics
Table C2: Bronchodilator Spirometry Results
Table C3: Spirometry values above and below normal limits as defined by GLI reference equations
Table C4: Univariate and multivariate regression results for abnormal lung function
Table C5: Univariate and multivariate regression results for reversible FEV$_1$

Figure D1: Box plot of the SES score by Employment Status
Figure D2: Box plot of the SES score by Income Category
Figure D3: Box plot of the SES score by Home Type
Figure D4: Box plot of the SES score by Education
Figure D5: Scatter plot of the SES score by Asset Sum
Table D1: The number of unsuccessful test results for each spirometry measure
Table D2: Review of mother’s with unsuccessful lung function tests
Figure D6: Histograms of model residuals for FVC GLI reference equation
Figure D7: Histograms of model residuals for FVC GLI reference equation
Figure D8: Histograms of model residuals for FVC GLI reference equation
Table D3: Observed and Predicted values for GLI reference equations
Figure D9: Scatter plot of residuals: FEV$_1$
Figure D10: Scatter plot of residuals: FVC
Figure D11: Scatter plot of residuals: FEV$_1$/FVC
Table D4: Multivariate regression results stratified by study site
A: PROTOCOL
1. Introduction

1.1 Background and justification

Chronic respiratory disease constitutes a substantial proportion of adult disease globally and in South Africa. Underdiagnoses and misdiagnosis of respiratory diseases remains a problem, with many patients going without accurate diagnosis and treatment. There is, in general, a scarcity of data from South Africa on local normative values and reference equations for adult lung function and predictors of lung function. Accurate and appropriate normal reference values allow for early detection of respiratory illness and perform an important role in monitoring respiratory health [13].

Respiratory diseases can be classified as either obstructive, restrictive or non-specific ventilator defect [15]. Restrictive lung disease implies a reduction in lung size and volume, making it difficult for people to fill and expand their lungs with air. Patients with obstructive lung disease either have damage to the lungs or suffer from a narrowing of the airways, making exhalation more difficult than normal. Common causes of respiratory disease are chronic obstructive pulmonary disease (COPD), asthma, bronchiectasis and bronchitis. There is a high prevalence of COPD and asthma in South Africa [2] [3], making these two respiratory ailments a significant public health problem.

1.2 Common Respiratory Diseases in South Africa: Asthma and COPD

Asthma

Asthma is a chronic inflammatory disorder of the airways. Chronically inflamed airways are hyper-responsive; they become obstructed and airflow is limited (by bronchoconstriction, mucus plugs and increased inflammation) when airways are exposed to various risk factors [4].

Risk factors for asthma include exposure to allergens (such as those from house dust mites, animals with fur, cockroaches, pollens and moulds), occupational irritants, tobacco smoke, respiratory (viral)
infections, exercise, strong emotional expressions, chemical irritants, and drugs (such as aspirin and beta blockers). Asthma exacerbations are episodic but airway inflammation is chronically present. [5]

Asthma is diagnosed on the basis of a patient’s symptoms, medical history and lung function. Reoccurring wheezing, coughing and difficulty breathing are key symptoms. Additionally the presence of atopic diseases such as eczema or hay fever can be suggestive of asthma. Spirometry testing is used to measure airflow limitation and reversibility in order to establish a diagnosis of asthma [6]. Diagnostic challenges for asthma are common. Patients might not display symptoms at every visit or may only display limited symptoms. It can also be difficult to distinguish asthma from COPD and often a trial of treatment is needed [3] [4].

**Chronic Obstructive Pulmonary Disease (COPD)**

COPD is a leading cause of death in the world and increases in its prevalence and mortality are predicted in the upcoming decades [7] [8]. Tobacco smoking is a major cause of COPD and South Africa has one of the highest rates of tobacco smokers in the world [2]. Additional causes of COPD apart from tobacco smoking include occupational dust and chemicals, indoor air pollution from biomass cooking and heating in poorly ventilated houses. [9]

GOLD provides a working definition of COPD as “Chronic Obstructive Pulmonary Disease (COPD) is a preventable and treatable disease with some significant extra pulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases” [9].

Although spirometry results do not fully capture the impact of COPD on a patient’s health, it remains the gold standard for diagnosing the disease and monitoring its progression. Spirometry is an essential tool for diagnosis and guidelines are tabulated by GOLD for use in classifying COPD [8].

Typical symptoms of COPD are chronic and progressive dyspnea, cough and sputum production.
Although often thought of as a disease of the elderly, recent studies have noted that in certain populations there is a high prevalence of COPD in younger adults [8].

**Asthma and COPD**

It is possible for patients to suffer from both asthma and COPD. Individuals with asthma who are exposed to noxious agents (such as cigarette smoke) are at risk for developing fixed airflow limitation. Usually it is possible to distinguish asthma from COPD, however if an individual has both chronic respiratory symptoms and fixed airflow limitation it may be difficult to differentiate between the two diseases [3] [9].

**1.3 Burden of COPD and Asthma in South Africa**

In South Africa, respiratory disease (excluding tuberculosis) was ranked as the seventh most important cause of disability adjusted life years in the year 2000 [10]. Asthma is not normally associated with a high mortality rate as it is often a well-controlled disease in developed countries. However in South Africa the disease is not always well controlled and in the year 2000 it was ranked as the 13\textsuperscript{th} top cause of death [11]. The case fatality rate in South Africa is reported as being the 5\textsuperscript{th} highest in the world with 18.5 per 100,000 asthmatics [12]. In the year 2000, COPD was responsible for 2.3\% of all deaths in South Africa [11], and a 2007 study by the BOLD collaboration noted the high prevalence of COPD in South Africa compared to other countries [16].

Due to difficulties with terminology and language barriers, self-reported rates of asthma and COPD often under estimate the true prevalence of chronic lung disease. In addition to this, specialised spirometry equipment is often only available in specialist facilities. This contributes to the low rates of diagnosis [11] and under reporting of the two diseases.

**1.4 Spirometry Diagnostics**

The term “spirometry” refers to common pulmonary function tests which measure the amount (volume) and speed (flow) of air that can be inhaled and exhaled. These tests are able to detect whether a patient is
suffering from abnormal lung function and respiratory illness. Airflow limitation is best measured by spirometry, and this is the most widely available and reproducible test of lung function.

The graph below displays a volume-time curve which shows the amount of air expired from the lungs as a function of time; this is a widely used diagram in the field of spirometry.

![Spirogram](image)

**Figure A2: Volume Time Curve [12]**

The figure above shows some of the common diagnostics provided by spirometry tests. These include Forced Vital Capacity (FVC) which is the total volume of air expired after a full inspiration. Forced Expiratory Volume in 1 Second (FEV₁) which is the volume of air expired in the first second during maximal expiratory effort, and the ratio of these two parameters, FEV₁/FVC which is the percentage of the vital capacity which is expired in the first second of maximal expiration [13].

The diagnostic tools provided by spirometry tests allow diagnosis of obstructive, restrictive or mixed respiratory abnormality. An improvement in FEV₁, FVC or both lung function outcomes after inhaled bronchodilator suggests the presence of reversible airflow obstruction. [6]. The results of spirometry tests assist in determining which lung disease a patient has, and allows a health care professional to determine which treatment should be followed.
1.5 The Use of Reference Equations in Pulmonology

Spirometry tests are a vital component in diagnosing and managing respiratory illness. In order to diagnose abnormal lung function, the results of spirometry tests are compared to predicted values, and lower and upper limits of normal (LLN and ULN), that are appropriate for the individual being tested [1].

Although there have been many papers on reference equations for spirometric indices, many of these are based on studies with small sample sizes [13]. Spirometry data from African countries is limited and there is a strong need for more data in this area. Many reference equations are also developed using a narrow age range which limits their relevance in a wider population [13] [1].

The Global Lung Initiative (GLI) was established in 2012 to address the need for global reference equations that are based on a sufficiently large and representative sample, across a wide age range and using current methodologies.

Global Lung Initiative

Following endorsement by the European Respiratory Society (ERS), the American Thoracic Society (ATS), the Asian Pacific Society of Respirology (APSR), the Australian and New Zealand Society of Respiratory Science (ANZSRS), the Thoracic Society of Australia and New Zealand (TSANZ) and the American College of Chest Physicians (ACCP), the Global Lung Initiative published the 2012 Spirometric Lung Function regression equations.

The GLI reference equations differ to normal linear spirometry reference equations as they incorporate a spline function which allows for a smooth fit over the entire age range. The reference equations consist of three parameters L, M and S which are functions of sex, age, height and ethnicity [1].

The GLI equations have proven to be a useful contribution to the field and the idea of globally applicable reference equations has been welcomed by health care professionals. However studies have indicated that the current equations still require work before they can be utilize on a global scale. There was little data
included from African studies which is problematic. Research is needed to determine the whether these reference equations are appropriate for use in a South African setting.

1.6 Aim

To describe lung function in healthy women and identify risk factors associated with abnormal lung function.

Objectives

1. To describe lung function in a group of healthy adult South African females enrolled in a birth cohort study, the Drakenstein Child Health Study (DCHS).
2. To compare lung function of the group to commonly used reference values, specifically to the Global Lung Initiative’s predicted values.
3. To describe the spectrum of abnormal lung function.
4. To investigate risk factors for abnormal lung function.

2. Methods

2.1 Research design

This cross-sectional analysis will use data collected by the DCHS.

The DCHS is a birth cohort study which comprises of 1000 mother-infant pairs. The mothers are enrolled at their 2<sup>nd</sup> antenatal clinic visit and infants are followed up with regular study visits until 5 years of age.

There is a sub-study within the DCHS which specifically investigates the mothers’ lung function, with spirometry results collected for all mothers during the study visit 6 to 10 weeks after birth. Although data about infants is also collected as part of the wider DCHS this particular study will focus exclusively on data collected from mothers.
2.2 Study site, population, and sampling

The study is located in the Drakenstein area of the Western Cape, South Africa. It is a peri-urban area and the study population has a relatively low socioeconomic status. There are two main recruitment areas with primary health clinics, Mbekweni has a predominantly Black South African population and TC Newman is a predominantly mixed race community. A number of study visits, and the lung function testing, happen at Paarl Hospital which is in close proximity to both TC Newman and Mbekweni.

All mothers attending their 2nd antenatal clinic visit at TC Newman or Mbekweni are invited to join the DCHS. The only screening requirements are that they are over the age of 18, between 20 – 24 weeks pregnant and are planning to stay in the area after the birth of their child.

The sample size for this Maternal Lung Function paper would be determined by the number of mothers who have had their 6-10 week visit with lung function testing. There were 462 women in total who had data for their lung function test. A post-hoc power calculation was done to determine whether this study will be adequately powered to meet its objectives, Appendix A1.

2.3 Data collection

The Maternal Medical and Respiratory Enrolment, Maternal Lung Function and Maternal SES case report forms (CRFs) were used for this study (Appendix D8).

Apart from the clinical CRFs listed above, certain lab results will also be included in the analysis. A number of specimens are collected; one of them is urine which is requested at the enrolment visit and birth visit. Urine specimens were used in a quantitative analysis of urine cotinine (IMMULITE 200 Nicotine Metabolite, Siemans, Los Angeles, USA) to determine a mother’s smoking status. Smoking exposure based on urine cotinine was defined as: active smoker if urine cotinine >500 ng.ml-1; passive smoker if urine cotinine 10-500 ng.ml-1 and non-smoker if urine cotinine <10 ng.ml-1 [17].
Where a mother’s HIV status is unknown or not recently been tested, a blood specimen was taken and serum used in an ABON™ HIV 1/2/O Tri-Line HIV Rapid Test or alternatively an ADVANCED QUALITY™ Rapid Anti-HIV(1&2) Test HIV test. Serum from the blood specimens was also used in a multiple-allergen Phadiatop® test (UniCAP®-Pharmacia, Sweden) which has shown to be a satisfactory method for screening for atopy, with a level greater than > 0.35 ku/l regarded as atopy.

2.4 Data management

CRFs are captured into a Microsoft Access database which is structured as a relational database according to the Participant identification (PID) number. All data is stored according to the PID number to ensure participant confidentiality. There are various checks to minimize data capturing errors and a dedicated effort to control the quality of the data.

Urine cotinine results are used to determine maternal smoking status. These results are made available to the Drakenstein study on a lab report form which is also stored in the relational Access database. HIV status is collected on the Maternal Medical and Respiratory Enrolment CRF. Atopy results are stored in a Microsoft Excel spreadsheet with the PID and results, both are checked thoroughly for quality control issues. These results are merged together with the data from the study database.

The database is stored in a secure network location, which is password protected with user rights. Backups are made on a regular basis and no participant names or personal identifiers are stored with the data. Data is exported from the database into excel and then into Stata where tables are merged and cleaned for statistical analysis. Data collection for the DCHS began in 2013 and is ongoing. Data management and manipulation is needed to create a dataset suitable for this study.

2.5 Data analysis

The lung function results are only available for a single visit and the analysis will thus be cross-sectional in nature.
To describe the lung function of the cohort the medians and interquartile ranges of the following spirometry results will be presented: FVC and FEV₁ and the ratio FEV₁/FVC.

The GLI reference equations will be fitted to the observed data and the model fit assessed by a statistical analysis of the model residuals. The GLI predicted values will be compared to the observed values to determine abnormal lung function. Reversible lung abnormalities will be defined by a 12% or greater increase in FEV₁ after bronchodilator. The z scores generated by the GLI reference equations will also be considered.

A multivariate logistic regression model will be fitted to explore risk factors associated with abnormal lung function and reversibility. The following clinical covariates of interest will be included in the analyses: HIV status, previous / current TB infection, tobacco usage (as measured by urine cotinine), medical history, atopy profile and BMI. Socioeconomic information will also be included as a covariate of interest. Those covariates with are significant at the 5% and 10% level will be considered in the multivariate model.

Analysis will be done in STATA version 12.

### 2.6 Ethical issues

The ethical principles will be in line with those stated in the Declaration of Helsinki (Fortaleza, Brazil, 2013) [14], a declaration developed by the World Medical Association for medical research involving human participants.

The study has been designed taking into account the social and clinical value of the research objectives. It is accepted that all research projects carry some element of risk and inconvenience for participants. In light of the potential value that this study could generate and given the burden of disease in South Africa, and notable lack of data in this field, the potential risks associated with this study are considered reasonable. Importantly the study aims to minimize risks to participants and ensure that their well-being
takes precedent over all other interests at all times. The welfare of study participants will be closely monitored and any participants requiring additional medical attention or counselling will be referred to an appropriate health care professional.

The study is considered to have a high level of scientific validity, it has been designed in line with similar international studies and has a team of lung function experts using suitable spirometry equipment and following best clinical research practices.

Participants will have full autonomy to choose what activities within the study they would like to participate in, and their decisions will be free from any coercive influence. Before study activity commences, participants will be taken through an informed consent process where there will be full disclosure about the nature of the study, the risks, benefits and alternatives and an opportunity for participants to ask questions. All study materials and CRFs will be translated into mother tongue languages of the participants. Participants will also be able to withdraw from the study at any time with no penalties.

There will be equitable selection of participants. All mothers attending an antenatal clinic visit at one of the clinics in Paarl are invited to participate in the study, and these mothers come from communities that are likely to benefit directly from the findings of the study.

Participants’ privacy and confidentiality will be well protected. All data will be stored confidentiality and using participant identification numbers instead of participant names. Participants files will be kept in a locked room and sensitive results and data will be treated and stored carefully.

3. Time frame
The DCHS study began enrolling mothers in March 2012 and a large amount of data collection, capturing and cleaning has already taken place. As such this time frame focuses on the critical milestones required for the data cleaning and analysis of Maternal Lung Function data.
### Table A1: Timeframe for study schedule for maternal lung function analysis

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### 4. Dissemination of research findings

This thesis will be submitted in partial fulfilment of the requirements for a Master of Public Health (Epidemiology) degree at the University of Cape Town. The results and findings fall within the organisation of the DCHS, findings will be discussed with and presented to the lead investigator Heather Zar and study epidemiologist Landon Myer.
Appendix A1: Post-hoc power calculation

The sample size for this Maternal Lung Function paper is determined by the number of mothers who have had their 6-10 week visit with lung function testing, there were 462 women in total who had data for their lung function test.

Given that the sample size for this study is fixed, a post-hoc power calculation was done to determine the study’s ability to correctly detect a significant effect with respect to mean differences in the spirometry results compared to the GLI predicted spirometry values. The observed mean FEV\(_1\) value was 2.72 L (sd 0.44) with a GLI mean predicted value for this cohort of 2.80 L (sd 0.27), this provides a power of 0.91. Similarly for FVC with an observed value of 3.15L (sd 0.40) and GLI predicated value of 3.23 L (sd 0.32) the statistical power is 0.92. Thus we believe the study is adequately powered to meet its key objectives.
 References


B: LITERATURE REVIEW
1. Introduction and objectives of this literature review

This literature review provides an overview of the burden of adult respiratory diseases in lower and middle income countries (LMICs) and specifically in South Africa. It will review the most common respiratory diseases with a specific focus on COPD and Asthma. It will explore measurement and diagnostics tools used in the field of pulmonology. Lastly the use of reference equations and the Global Lung Initiative are discussed.

2. Search Strategy

PubMed and Google Scholar were used to find papers for this literature review. The following search terms were used: asthma, chronic obstructive lung disease, respiratory illness, spirometry, lung disease, Global Lung Initiative, pulmonology, reference equations, lung cancer, tuberculosis, Human Immunodeficiency Virus, low and middle income countries and South Africa. The literature review focused specifically on papers that looked at lung disease and included spirometry data or used spirometry reference equations.

The search focused on papers from the African continent but also included those written globally. All publications available through to 16 February 2015 were included in this review, and only English language articles were included.

3. The Burden of Adult Lung Disease in Low to Middle Income Countries

Historically, low and middle income countries (LMICs) have had a high burden of infectious diseases and health care budgets and facilities have been focused on responding to these epidemics. In recent years more attention has been given to the non-communicable disease (NCD) burdens faced by LMICs, including diseases related to respiratory illness.

Respiratory diseases are classified as either obstructive or restrictive. Patients with obstructive lung disease either have damage to the lungs or narrowing of the airways which makes exhalation more
difficult than normal. Obstructive disease is considered to be either reversible (for example asthma) or only partially reversible or entirely irreversible (for example chronic obstructive lung disease). A restrictive abnormality is defined by a reduction in total lung capacity with submaximal inspiratory or expiratory efforts, or poor peripheral airflow obstruction. Restrictive lung diseases are rarer than obstructive respiratory illnesses. Asthma and COPD cause significant burden of disease in LMICs, other significant chronic respiratory conditions are tuberculosis (TB), HIV associated lung disease and lung cancer.

The global burden of asthma has been estimated to be approximately 623 million people of all ages and ethnic backgrounds worldwide who suffer from this chronic respiratory illness [15]. The World Health Survey (WHS) was developed and implemented by the WHO in 2003. This study surveyed a total of 178,215 individuals from 70 countries aged 18 to 45 years. Results from this study indicated that the global prevalence rates of doctor diagnosed asthma, clinical/treated asthma and wheezing in adults were 4.3%, 4.5%, and 8.6% respectively [15]. The highest prevalence rates were observed in resource rich countries, however the authors note that many resource poor African nations also have a high prevalence of the disease but diagnosis rates are low [15]. The burden of asthma is high in urban settings and non-affluent countries. In these settings, under diagnosis and under treatment remains a problem [16]. There are more published research papers on the prevalence and risk factors for asthma in children than in adults, and more research is needed in this area, especially in low income settings. Existing research has noted that there is an increasing burden of asthma in LMICs and intensified strain on health services to provide on-going treatment and care [17]. In 2004 it was estimated that the number of disability-adjusted life years (DALYs) lost due to asthma worldwide was approximately 15 million per year [12]. Asthma accounted for around 1% of all DALYs lost worldwide which is reflective of the high prevalence and severity of asthma [12].
COPD is globally associated with high rates of morbidity and mortality [18]. The burden of disease for COPD is high and it has previously been ranked twelfth as a worldwide cause of lost quality and quantity of life [18]. A study by the World Health Organisation projected that COPD might rank fifth by the year 2020 as a leading cause of chronic morbidity and mortality [9]. COPD is a leading cause of mortality in LMICs [2] [7]. A systematic review by Finney et al. noted that more population-representative studies using appropriate case definitions are needed in Africa to inform prevention and management strategies [19].

One recent African study, the FRESH AIR study in Uganda, investigated the prevalence and risk factors of COPD. The study found the prevalence of COPD in Uganda was highest in people aged 30 – 39 years (38% of men and 40% of women), this is unlike in European countries where there are substantially more cases in older adults. This reflects the difference in absolute numbers as the populations in Europe are older in comparison, in Africa a larger portion of the population are young adults [20]. The study noted that in the rural districts in Uganda, COPD starts early in life [8] and there was a notable burden of disease in young adults.

The Burden of Obstructive Lung Disease (BOLD) study investigated COPD prevalence in 12 study sites around the world. Prevalence of stage 3 to 4 COPD ranged from 0.8% in Germany to 6.7% in South Africa [2]. The study noted that although smoking is still a strong risk factor for the disease, there were other notable risk factors such as prior tuberculosis and occupational exposures [2].

In 2011 it was estimated that there were 8.7 million new cases of TB, TB killed 1.4 million people and rates of disease were especially high in parts of Africa [16]. TB disproportionately affects people living below the poverty line and is especially prevalent in LMICs [21]. The economic burden of TB care is often very high this, and the difficulties with ongoing treatment programmes, causes significant problems in many LMICs [21]. Adults who have previously been infected with TB, or who are currently infected with TB are likely to have chronic chest symptoms and lung function loss [22]. Ehrlich et al. noted that
combined obstructive/restrictive lung function loss was the most common functional outcome for patients with pulmonary tuberculosis, with a net obstructive effect on their lung function [22].

Infection with HIV makes the lungs more susceptible to a wide array of infectious and non-infectious diseases. Before widespread antiretroviral therapy (ART) treatment, HIV-infected adults often faced serious complications with acute bronchitis, bacterial pneumonia, TB, pneumocystis pneumonia and Kaposi’s sarcoma [23]. In recent years, with early ART therapy the incidence of opportunistic and recurrent infections has decreased, however there has been a subsequent increase in chronic respiratory disease associated with HIV infection [23].

There is a high burden of respiratory disease amongst HIV infected persons and an association between HIV and COPD has been noted in a number of studies [23] [24]. Calligaro and Gray note that there is a level of synergism between viral factors, opportunistic infections, conventional influences like tobacco smoke and biomass fuel exposure, and possibly, the immunological effects of ART on a patient’s risk of developing HIV-associated chronic obstructive lung disease [23]. However they also note that there is a scarcity of data exploring these associations and more research is needed in this area.

The main spirometric abnormality associated with HIV infection in adults is a low FEV$_1$ and/or a low FEV$_1$/FVC ratio, which is generally indicative of non-reversible obstructive lung disease [23]. There is a strong association between age and the presence of obstructive lung disease, and it is unusual to see a high prevalence of obstructive lung disease in young adults. Thus most studies that have shown a higher prevalence of obstructive lung disease in HIV infected adults have been based on older cohorts where the mean age is often above 40 years of age. [23]

Lung cancer was one of the most commonly diagnosed cancers worldwide in 2008 with an estimated 1.61 million cases and 1.38 million deaths [25]. The majority of lung cancer cases (55%) take place in LMIC countries, this is an increase compared to previous years where only 31% of lung cancer cases were attributed to high income countries [25].
4. Common Respiratory Diseases in South Africa

Chronic respiratory diseases account for a large proportion of NCDs in South African adults. Underdiagnoses and misdiagnosis of respiratory diseases remains a problem, with many patients going without proper diagnosis and treatment [3]. Asthma and COPD have been reported to be amongst the most common respiratory illnesses in adults in South Africa, making these two respiratory ailments a significant public health problem [3] and the focus of this section of the literature review.

4.1 Asthma

The prevalence of asthma and other atopic diseases has been increasing worldwide in recent years. Evidence suggests that the rate of asthma increases as communities become more urbanised and adopt western lifestyles [12]. Respiratory disease as a group, excluding TB, was ranked as the seventh most important cause of Disability adjusted life years (DALYs) in 2000 in South Africa, with 4.7% of all DALYs attributed to respiratory illness [10]. Asthma is largely an adequately controlled disease in developed countries and not often a top cause of mortality. In South Africa, asthma ranked as the 13th top cause of death (1.5% of all deaths) and 18th as a cause of life years lost (0.9% of all life years) in the year 2000 [10]. Although South Africa is ranked 25th worldwide in asthma prevalence with an estimated 8.1% prevalence over all ages, it is ranked 4th worldwide in the asthma mortality rate in the 5 – 34 year age group [12]. Additionally the asthma case fatality rate in South Africa is reported as being the 5th highest in the world with 18.5 per 100,000 asthmatics [12].

It was noted in the South Africa demographic health survey of 2003 that self-reported rates of asthma are often an unreliable guide to the true prevalence of asthma [11]. Sometimes there is confusion around the terminology used, especially when terms are translated from English to one of the other South African National languages. Additionally, spirometric lung function testing is essential for proper diagnosis of respiratory illness and is often only available in specialist facilities. Under diagnosis of asthma is also a well-documented phenomenon and can add to the low rates of self-reported asthma [11].
Asthma is diagnosed on the basis of a patient’s symptoms, signs and lung function testing [4]. Spirometry is the preferred method of measuring airflow limitation and reversibility and it is generally used to establish a diagnosis of asthma. An increase in FEV$_1$ of more than 12% (or greater than 200L) after administration of a bronchodilator is suggestive of reversible airflow limitation, consistent with asthma if it is fully reversible. Other studies have also suggested that improvements in both FEV$_1$ and FVC should be considered for a diagnosis of reversibility [5]. Because many asthma patients don’t demonstrate reversibility at each assessment, repeated testing is advised [6]. Airway reversibility, especially partial reversibility is not however specific to asthma and may also be a feature of other chronic respiratory diseases such as COPD.

There are various diagnostic challenges when it comes to diagnosing asthma. Some patients with asthma have a chronic cough as their only symptom. Other patients might only suffer from exercise induced bronchoconstriction. Distinguishing asthma from COPD can be difficult and may require a trial of treatment [4]. Undiagnosed asthma remains a significant problem with many asthmatic patients going without proper diagnosis and treatment [26]. Many patients with decreased lung function remain undiagnosed because they aren’t symptomatic at the time of their appointment [26]. Additionally patients who do present with respiratory problems and who do have reduced lung function are not always recognised as being asthmatic [26]. It would also appear that the patient’s perception of dyspnoea and their ability to perceive their own dyspnoea plays a vital role in their discussions with their health care practitioner and subsequent diagnosis of asthma [26].

There have been a number of papers which highlight the problematic relationship between smoking and asthma, evidence suggests that active smokers, and particularly females, are at a higher risk for developing asthma than non-smokers [27] [28]. Smoking status and smoking duration are strongly related in a “dose-dependent fashion” to the extent of asthma severity. Asthmatic smokers are at risk of developing more severe problems, with a higher frequency of exacerbations and a higher number of life threatening asthma attacks [27].
Lung function is considered to be at its maximum around 20 to 25 years of age. After the age of 25 it begins to decline with annual declines in FEV\textsubscript{1} of approximately 25-30L per year [28]. However the estimated rate of decline is not linear with age and is often greater in elderly patients. Age and the number of years for which a patient has been smoking interact to form a strong risk factor for development of asthma in adulthood [28].

The association between obesity and asthma has been observed in a number of studies [29] [30], there are a number of hypotheses for this association; these are mostly based on mechanical, inflammatory and common genetic risk factors [29]. There have been few studies specifically investigating the relationship between body weight and asthma among reproductive age women. One study examined the relationship between asthma and pre-gravid obesity among reproductive aged women. They found a significant association where the odds of asthma were 1.5 fold among women who were overweight or obese [29]. Additionally they noted that adult diagnosed asthma was positively associated with a weight change of more than 20kgs [29].

Results from the National Health and Nutrition Examination Survey (NHANES), 2001-2004 [31], found that extreme obesity and smoking were strongly associated with current asthma or ever been diagnosed with asthma in both women and men [31]. In this study approximately 20% of extremely obese women and men had ever been diagnosed with asthma, and within this group of individuals with high BMI, 15% reported that they currently had asthma, these rates were much higher than in the normal weight population [31].

**Chronic Obstructive Pulmonary Disease (COPD)**

In South Africa, in 2003, COPD was responsible for 2.3% of all deaths, although only 1.1% of years of life lost, which can be attributed to the fact that it is primarily a disease of the elderly [10]. Correct diagnosis of COPD requires specialised spirometry equipment and this contributes to the current high rates of under-diagnoses of the disease in Africa [32]. The BOLD study noted that the study site in South
Africa had the highest prevalence of stage two or greater COPD, and that the sites in South Africa, Philippines and USA had a much higher than usual burden of clinically significant COPD than other countries in the study [2].

Similarly to asthma, self-reported rates of COPD can be unreliable and can underestimate the true prevalence of chronic lung disease. There is often confusion around the terminology used for COPD, for example a patient might be told that they have “emphysema” as opposed to “chronic obstructive lung disease” or “COPD” [5].

Although spirometry results do not fully capture the impact of COPD on a patient’s health, it remains the gold standard for diagnosing the disease and monitoring its progression [9]. Spirometry is an essential tool for diagnosis and the following specific cut-points are tabulated by GOLD for use in classifying COPD.

**Table B1: Spirometric Classification of COPD Severity** [9]

<table>
<thead>
<tr>
<th>Spirometric Classification of COPD Severity based on Post-Bronchodilator FEV</th>
<th></th>
</tr>
</thead>
</table>
| Stage 1: Mild | FEV$_1$/FVC < 0.70  
FEV$_1$ ≥ 80% predicted |
| Stage 2: Moderate | FEV$_1$/FVC < 0.70  
50% ≤ FEV$_1$ < 80% predicted |
| Stage 3: Severe | FEV$_1$/FVC < 0.70  
30% ≤ FEV$_1$ < 50% predicted |
| Stage 4: Very Severe | FEV$_1$/FVC < 0.70  
FEV$_1$ < 30% predicted |

Typical symptoms of COPD are chronic and progressive dyspnea, cough and sputum production. For many patients, chronic cough and sputum production may precede the development of airflow limitation by many years [9]. COPD was previously considered to be a disease of the elderly and not often seen in
young adults. Recent studies have noted that COPD can start earlier in life, in the FRESH AIR study in Uganda there was a substantial prevalence of COPD in young adults [8].

Tobacco smoking is a major cause of COPD and one of the most widely recognised risk factors for COPD [33] [34]. Many studies have only focused on tobacco smoke when researching COPD, but in recent years other risk factors have come to light. Other potential risk factors for COPD include occupational dust and chemicals, air pollution (potentially from indoor biomass cooking or heating in poorly ventilated houses), airway hyper responsiveness, asthma, and certain genetic variations [5] [33].

Of relevance to South Africa is the fact that many studies have noted biomass smoke as a primary risk factor for COPD in rural areas [18] [34], exposure to biomass smoke was significantly associated with COPD (odds ratio 2.3 95% CI 1.5 – 3.5), acute respiratory tract infection (OR 3.64, 95% CI 2.1 – 6.4) and wheeze (OR 2.1, 95% CI 1.5 – 2.9) [35].

Pulmonary TB has shown to be associated with chronic airflow obstruction, and specifically COPD at the time of diagnosis, during treatment and several years after treatment has ended [34] [5]. A nationwide South African study suggested that the strongest predictor of COPD was a history of pulmonary TB with OR 4.9 (95% CI 2.6 – 9.2) for men and OR 6.6 (95% CI 3.7 – 11.9) for women [5]. In this study, the risk of COPD with TB was stronger than the risk from tobacco smoking or exposure to smoke from biomass fuel [5].

COPD was previously considered a disease that affects mainly men, but in recent years there has been a rapid increase in the prevalence, morbidity and mortality of COPD in women [18] [36]. It has been suggested that the increase in tobacco consumption among women is the reason for the higher rates of COPD.

The table below notes the differences between the onset, etiology and airflow limitation of Asthma and COPD.
Table B2: Comparison of asthma and COPD

<table>
<thead>
<tr>
<th></th>
<th>Asthma</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>Onset normally early in life</td>
<td>Onset normally midlife, symptoms usually begin at &gt;40 years of age</td>
</tr>
<tr>
<td>Etiology</td>
<td>Possible family history of allergies or asthma</td>
<td>Long smoking history or history of exposure to environmental pollutants</td>
</tr>
<tr>
<td>Airflow Limitation</td>
<td>Reversible</td>
<td>Not fully reversible</td>
</tr>
</tbody>
</table>

5. Spirometry

Spirometry is the most widely used method for measuring lung function in adults. Spirometry can provide critical information about the type and reversibility of lung disease including on the large and small airways and the pulmonary parenchyma [37]. It is important to note that the results of a spirometry test do not provide an exact diagnosis; rather they provide insight which allows the pulmonologist to compare a patient’s lung function to different patterns of abnormalities, this process assists in diagnosing a specific lung function disorder.

Guidelines for performing and interpreting spirometry are published by a number of different organisations, including the ERS and the ATS. Good standardization for performing testing is essential and tests are very dependent on the effort put in by the patient, thus patient cooperation and awareness is vital in obtaining optimal results. Unsuccessful spirometry with suboptimal results may occur in patients who have some clinical difficulty (such as chest or abdominal pain) or with patients who do not fully understand the directions given to them when performing the test [37]. Patient understanding is especially important in health care settings in countries like South Africa. There can be a language barrier between the health care professional and the patient, and this can lead to poorly understood instructions and ultimately suboptimal test results. Spirometry tests are performed a minimum of three times to ensure that the results are reproducible and accurate [37] [6].
In order to determine whether a particular patient has impaired lung function ability, the patient’s results will be compared to normal or predicted ranges of values that are obtained from large population studies of healthy subjects [37] [1] [6]. Measurements are compared to patients with a similar age, height, sex and where appropriate ethnicity.

There are a number of common diagnostics or parameters provided by spirometry tests. These include the following:

FVC -- This is the total volume of air expired after a full inspiration. Patients with obstructive lung disease usually have a normal or only slightly decreased vital capacity. Patients with restrictive lung disease have a decreased vital capacity.

FEV₁ -- This is the volume of air expired in the first second during maximal expiratory effort. FEV₁ is reduced in both obstructive and restrictive lung disease.

FEV₁/FVC -- This is the percentage of the vital capacity which is expired in the first second of maximal expiration. In healthy patients FEV₁/FVC is usually around 70% or above the lower limit of normal (as defined by appropriate reference equations). In patients with obstructive lung disease FEV₁/FVC decreases and can be as low as 20-30% in severe obstructive airway disease. Restrictive disorders have a near normal FEV₁/FVC [13].

Spirometry tests assist in diagnosing ventilatory defects. Patients may suffer from an obstructive, restrictive or mixed abnormality. An obstructive ventilatory defect is a reduction of maximal airflow from the lung in relation to the maximal volume that can be displaced from the lung. It is suggestive of airway narrowing and is defined by a reduced FEV₁/VC ratio below the 5th percentile of the predicted value [5]. A restrictive ventilatory defect is characterised by a reduction in total lung capacity below the 5th percentile of the predicted value, and a normal FEV₁ / VC ratio [5]. A mixed ventilatory defect is characterised by the coexistence of obstruction and restriction, and is defined physiologically when both FEV₁/VC and total lung capacity are below the 5th percentiles of their relevant predicted values [5].
6. The Role of Reference Equations in Spirometry

Spirometry tests play a critical role in diagnosing and managing respiratory illness. Unlike many biological indices which often have specific threshold values, pulmonary function typically varies with age, standing height, sex and ethnicity. This necessitates that the results of lung function tests are compared to predicted values, and lower and upper limits of normal (LLN and ULN), that are appropriate for the individual being tested [1] [37].

There are a large number of published reference equations for adult pulmonary function, mostly for spirometric indices. Many of these reference equations are based on studies with small sample sizes, with data collected many years ago and with an unrepresentative sample for the greater population [1]. Spirometry data for Black Africans is particularly scarce and most of the reference equations are based on studies of Caucasian participants. Many prediction equations also were designed for a specific age group, which can lead to significant discontinuities as individuals move from one set of equations to another [1]. Many health care professionals working in pulmonology use the default settings offered by the spirometry equipment’s manufacturer and they are not aware of these shortcomings [55].

There is, in general, a scarcity of data from South Africa on normative values and reference equations for lung function, predictors of low lung function and findings on the impact of HIV on adult lung function [3]. Accurate and appropriate normal reference values are needed as they allow for early detection of respiratory illness and perform an important role in monitoring respiratory health.

The GLI was established in 2012 to address the need for global reference equations that are based on a sufficiently large and representative sample, across a wide age range and using current methodologies.

7. The Global Lung Initiative

The GLI published the 2012 Spirometric Lung Function regression equations based on data collected from the following countries: Algeria, Australia, Austria, Brazil, Canada, Chile, China, France, Germany, Iceland, India, Iran, Israel, Italy, Mexico, the Netherlands, Norway, Oman, Pakistan, Philippines, Poland,
Portugal, South Africa, South Korea, Sweden, Switzerland, Taiwan, Thailand, Tunisia, UK, USA, Uruguay and Venezuela. There were various data quality control issues that meant some data was not useable, for example, data was discarded if there were missing values for sex, age, height, FEV$_1$ or FVC, or where the FEV$_1$/FVC ratio was $>1.0$ [1]. Additionally, datasets from India, Pakistan, Iran, Oman, the Philippines and South Africa were either too small in number for analysis, or could not be combined into groups with other sets (due to a high level of heterogeneity) and these were thus excluded from the reference equation analysis.

There were significant differences for spirometric indices between countries, a regression analysis by the GLI team highlighted that there was remarkable agreement between some of the datasets but predicted values for FEV$_1$ and FVC were significantly lower in other groups. There was no evidence to suggest that these differences related to methodological differences or to unrepresentative samples.

From this the following groups were defined:

**Group Country/region**

‘Caucasian’  Europe, Israel, Australia, USA, Canada, Mexican Americans, Brazil, Chile, Mexico, Uruguay, Venezuela, Algeria, Tunisia

‘Black’  African American

‘South East Asian’  Thailand, Taiwan and China (including Hong Kong) south of the Huaihe River and Qinling Mountains

‘North East Asian’  Korea and China north of the Huaihe River and Qinling Mountains

The table below displays the differences in the level of pulmonary function between participants in each of the groups compared to Caucasians.

**Table B3: Percentage difference in mean pulmonary function by sex and ethnic group compared to Caucasians [1]**

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEV$_1$</td>
<td>FVC</td>
<td>FEV$_1$/FVC</td>
<td>FEF$_{25-75%}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Quanjer et al. suggested that the proportional differences in the level of pulmonary function between Caucasians, South and North East Asians and African-Americans are compatible with the “out of Africa” theory [1]. This theory proposes that mankind originated in Africa and migrated through South East Asia, travelling northwards and populating East Asia whilst undergoing evolutionary changes [1], with a hypothesis that as people grew taller their lung volumes increased proportionately. There is evidence from genetic markers which supports the “out of Africa” theory” [38].

It is suggested that the ethnic and racial differences in pulmonary function arise from differences in body build (specifically the chest size or the ratio of sitting to standing height), socioeconomic status, living in an area of high altitude and other potential environmental factors [39] [40] [41]. It is however widely accepted that further research is needed into the interaction effect between ethnic group and pulmonary function, especially for minority groups [39].

The GLI reference equations include an “other” group for individuals not represented by the four GLI groups, or for individuals of mixed ethnic origins. This “other” group is a composite equation which takes the average of the other equations, meaning that individuals in this “other” group are compared to the average of the four main ethnic groups. This group is currently in place to facilitate interpretation and discussion until a more appropriate solution is developed [39].
The GLI equations

These equations differ slightly from usual spirometric reference equations (which are usually based on linear regression models) in that they incorporate spline functions to allow the dependent variable to have a smooth and non-linear function. This is important for a smooth fit over the entire age range rather than with previous references equations which led to large jumps between small incremental increases in age.

The GLI reference equations have three parameters L, M and S which are functions of sex, age, height and ethnicity.

- L measures the skewness
- S is the coefficient of variation
- M is the predicted value of FEV\textsubscript{1}, FVC, FEV\textsubscript{1}/FVC or other indices

The general form of the equation is given by:

\[ Y = a + b \times H + c \times A + \text{age-spline} + d_1 \times \text{group} + d_2 \times \text{group} \times A \]

- \( Y \) = dependent variable
- \( H \) = standing height (cm)
- \( A \) = age (yr)
- \( a, b, c, d_1 \) and \( d_2 \) are coefficients which vary for each ethnic group
- \( \text{spline} \) is an age-specific value from the spline function
- \( \text{group} \) is a dummy variable, with 0 for Caucasians and 1 otherwise

Because the reference equations incorporate spline functions the interpretation of the parameters is not as simple as with a normal linear regression equation. An example of the workings is given in Appendix B1.

The current GLI equations are a good start at moving towards globally appropriate prediction equations and the idea of globally applicable reference equations has been welcomed by health care professionals.
However studies have indicated that the current equations still require work before they can be utilized on a global scale. Researchers working with the GLI were not able to gather data for many ethnic minorities or any African countries. There was very little data available from African studies that could be used in the reference equation analysis. The reference equations available for Black individuals in the GLI equations are based on data from African Americans. Thus, while the GLI reference equations provide important insight into lung function analysis, there is an urgent need for reference data and equations that are appropriate for African communities.
References


Appendix B1: GLI Reference Equations

The table below is provided by the GLI Macro and shows the parameters used to calculate L, mu and sigma for FEV₁.

<table>
<thead>
<tr>
<th>Table B4: Example parameters for the fields L, mu and sigma used in the GLI reference equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>log link</td>
</tr>
<tr>
<td>log ht</td>
</tr>
<tr>
<td>coef int</td>
</tr>
<tr>
<td>Height cm</td>
</tr>
<tr>
<td>coef age</td>
</tr>
<tr>
<td>power age</td>
</tr>
<tr>
<td>Afr. Am.</td>
</tr>
<tr>
<td>NE Asia</td>
</tr>
<tr>
<td>SE Asia</td>
</tr>
<tr>
<td>O/M</td>
</tr>
</tbody>
</table>

The GLI reference equations have three parameters L, M and S which are functions of sex, age, height and ethnicity.

- L measures the skewness
- S is the coefficient of variation
- M is the predicted value of FEV₁, FVC, FEV₁/FVC or other indices

The general form of the equation is given by:

\[ Y = a + b*H + c * A + \text{age-spline} + d_1 * \text{group} + d_2 * \text{group} * A \]

- Y = dependent variable (may be log transformed)
- H = standing height (cm) (may be log transformed)
A = age (yr) (may be log transformed)
a, b, c, d₁ and d₂ are coefficients which vary for each ethnic group
spline is an age-specific value from the spline function
group is a dummy variable, with 0 for Caucasians and 1 otherwise

One can see from the table that the coefficients for the predicted values of FEV₁ for Females would be: -0.148 for African Americans, -0.015 for North East Asians, -0.121 for South East Asians and -0.071 for participants in the “Other” category.
C: MANUSCRIPT
Lung Function in Healthy Adult South African Females

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Keywords: Maternal Lung Function, Spirometry, South Africa, Global Lung Initiative

*As per the MPH dissertation guidelines, co-authors are not listed on the journal ready manuscript. The contribution of supervisors is noted in the acknowledgments section of this dissertation. This article is written according to the requirements in the Instructions for Authors for the Journal of the Respiratory Research. These instructions are included in Appendix D6
Abstract

Background: Accurate and appropriate spirometry reference values allow for early detection of respiratory illness and perform an important role in monitoring lung health. There is, in general, a scarcity of data from Africa, and the Global Lung Initiative (GLI) has published global reference equations but models did not include data from African studies. The aim of this study was to investigate lung function in a group of healthy South African females and the applicability of the GLI reference equations.

Methodology: Maternal lung function testing was undertaken at 6 to 10 weeks post-partum as part of a birth cohort study, the Drakenstein Child Health Study. Pre- and post-bronchodilator spirometry was performed according to a standardised protocol and correlated with clinical information. Bronchodilator response was assessed by repeating spirometry 15 minutes after administration of inhaled 400mcg salbutamol.

Results: A total of 462 women were included, mean age 17 years (range 18 – 42 years). The GLI reference equations fitted the observed lung function results well for the group of mothers who did not self-report smoking or asthma. There were 64 (14%) mothers with an abnormal Forced Expiratory Volume in 1 Second (FEV₁) result, 60 (13%) mothers with an abnormal Forced Vital Capacity (FVC), and 35 (8%) mothers with an abnormal FEV₁/FVC ratio. There were 22 (5%) mothers who had reversible FEV₁; the rate of undiagnosed reversibility was 4% of the cohort. High body mass index was associated with a higher risk for poor FVC and FEV₁/FVC lung function, OR 1.40 (CI: 1.01, 1.65) and OR 1.25 (CI 1.10, 1.95) respectively. Mothers with a higher socio-economic status had better FEV₁ with the adjusted SES OR 0.65 (CI 0.36, 1.08).

Conclusions: There was a high prevalence of abnormal lung function in this cohort of South African adult females and a number of cases of undiagnosed reversibility. Spirometry testing is important to diagnose lung disease in South African communities. The GLI’s reference equations were appropriate and applicable for a cohort of South African adult women.
1. Introduction

Respiratory illnesses constitute a large burden of disease in adults in lower to middle income countries including South Africa. Underdiagnoses and misdiagnosis of respiratory diseases remains a problem, resulting in suboptimal treatment. Respiratory disease as a group, excluding tuberculosis, was ranked as the seventh most important cause of disability adjusted life years (DALYs) in 2000 in South Africa, with 4.7% of all DALYs attributed to respiratory illness [10]. Asthma and Chronic Obstructive Pulmonary Disease (COPD) are two of the most common respiratory illnesses in adults in South Africa. Although South Africa is ranked 25th worldwide in asthma prevalence with an estimated 8.1% prevalence over all ages, it is ranked 4th worldwide in the asthma mortality rate in the 5 – 34 year age group [12]. The asthma case fatality rate in South Africa is reported as being the 5th highest in the world with 18.5 per 100,000 asthmatics [12]. In South Africa, in 2003, COPD was responsible for 2.3% of all deaths [10] Studies have noted the significantly high prevalence of COPD in South Africa and substantial burden of disease in the country compared to other LMICs [2].

In African populations the absolute burden of chronic lung disease can include a sizable number of cases in younger age groups. For instance, although COPD is normally associated with older patients, the FRESH AIR study in Uganda noted that there were a substantial number of cases in young adults and this age group contributed the most to the overall burden of disease [8]. African populations often have a greater percentage of the population who are young or middle aged adults with few over the age of 65 years [20]. There is a sizeable potential burden of disease in young African adults and this highlights the importance of spirometry tests for diagnosis in this group.

Several important risk factors for respiratory disease appear common in the South African population. Obesity and smoking have been found to be strongly associated with current asthma or ever been diagnosed with asthma and a more rapid decline in lung function [31]. Active smokers, and particularly females, are at a higher risk for developing asthma than non-smokers [27] [28]. The association between obesity and asthma has been observed in a number of studies [29] [30]. There are a number of hypotheses
for this association; these are mostly based on mechanical, inflammatory and common genetic risk factors [29].

Despite the burden of respiratory disease in South Africa, there have been few population-based studies employing rigorous measures of lung function. Spirometry tests play a critical role in diagnosing and managing respiratory illness. Pulmonary function typically varies with age, standing height, sex and ethnicity, and this necessitates that results of spirometry tests are compared to predicted values that are appropriate for the individual being tested [1] [37]. Although there are several published reference equations for spirometric indices, many of these are based on studies with unrepresentative samples [1]. Spirometry data for Black Africans are particularly scarce and most of the reference equations are based on studies that had participants of European descent and/or African-American populations [1]. Accurate and appropriate normal reference values that are applicable in a South African setting are urgently needed.

The aim of this study was to investigate lung function in an otherwise healthy group of South African females. The first objective was to describe lung function; the second was to compare the spirometry results of the group to the commonly used reference values provided by the Global Lung Initiative (GLI). The third and fourth objectives were to identify women with abnormal lung function and to investigate associated risk factors.

2. Methods

A cross-sectional analysis of lung function in women enrolled in the Drakenstein Child Health Study (DCHS) study was undertaken from March 2012 to June 2014.

2.1 Study setting and design

The DCHS is a birth cohort study conducted in a peri-urban, low socio-economic community in South Africa. The Maternal Lung Function study is a sub-study within the DCHS, where mothers are invited to
have spirometry tests done. DCHS is conducted in the Drakenstein Area in Paarl in the Western Cape, South Africa. Women in the study reside in two different neighbourhoods; the first (a predominantly mixed race area) is serviced by the TC Newman clinic and the second (a predominantly black African area) by the Mbekweni clinic. Inclusion criteria for the study were that women were older than 18 years, 20 – 24 weeks pregnant; intended to stay in the area for the duration of the study and willing to attend all study visits and gave informed consent.

2.2 Measurements

Case report forms

A number of case report forms (CRFs) were used for data collection; these are included in supplementary data, Appendix D8. These CRFs record demographic and anthropometric information as well as information about self-reported respiratory related diagnoses, history of diagnoses, and objective measures of past illness. A maternal socio-economic CRF provided information used to calculate the socio-economic score.

Clinical specimen measurements

Apart from the clinical CRFs, mothers were asked for blood and urine specimens. Urine specimens were used in a quantitative analysis of urine cotinine (IMMULITE 200 Nicotine Metabolite, Siemans, Los Angeles, USA) to determine a mother’s smoking status. Smoking exposure based on urine cotinine was defined as: active smoker if urine cotinine >500 ng.ml-1; passive smoker if urine cotinine 10-500 ng.ml-1 and non-smoker if urine cotinine <10 ng.ml-1[55]. Where a mother’s HIV status was unknown or had not recently been tested a blood specimen was taken and serum used in an ABON™HIV 1/2/O Tri-Line HIV rapid test or alternatively an ADVANCED QUALITY™ Rapid Anti-HIV(1&2) HIV test. Serum from the blood specimens was also used in a multiple-allergen Phadiatop® test (UniCAP®-Pharmacia, Sweden) which has shown to be a satisfactory method for screening for atopy, with a level greater than > 0.35 ku/l regarded as atopy.
Lung function measurements

Mothers performed spirometry according to a standardised protocol at 6 – 10 weeks post-partum at a study visit at Paarl hospital by trained research staff. Spirometry data were recorded using a Jaeger Masterscope spirometer (CareFusion, Switzerland). The volume signal of the equipment was calibrated once daily with a 3-L syringe. Tests were performed with the subject in a sitting position according to American Thoracic Society (ATS) / European Respiratory Society (ERS) guidelines [6][56]. The following measurements were recorded: Forced expiratory volume in 1 second (FEV$_1$), Forced vital capacity (FVC) and the ratio FEV$_1$/FVC. After oral instruction the subjects exhaled forcefully until three acceptable curves were obtained. The best estimates were selected for analysis; these estimates were those that were both robust and repeatable. The spirometry was repeated 15min after inhalation of 400 mcg of salbutamol given with a metered dose inhaler and spacer to assess response to bronchodilator therapy.

Quality was assured by ensuring equipment reliability and accuracy (daily calibration of volume calibration with a 3L syringe with accurate BTPS correction). All tests were done by 1 of 2 experience trained personnel and the first 50 spirometry results were independently reviewed by a pulmonologist to ensure quality was consistent. After that, results were reviewed by senior person only when a result was abnormal. Reproducibility and repeatability criteria in line with the ATS/ERS recommendations for spirometry testing were adhered to. Any test not meeting these criteria was excluded. Reversibility with respect to FEV$_1$ was defined as a 12% or greater difference between a mother’s pre-bronchodilator FEV$_1$ and her post-bronchodilator FEV$_1$ result. Post bronchodilator FEV$_1$ results were compared to the predicted values to assess whether reversibility was partial or full.

2.3 Data analysis

Data was captured into a DCLHS database in Microsoft Access and analysed using Stata 12.0 (Stata Corporation, College Station, USA).
Variables were inspected visually by means of histograms and box-plots. Descriptive statistics were used to summarize baseline characteristics. Bivariate associations were calculated using a Pearson’s chi-squared test (for categorical variables), a Wilcoxon rank sum test (for nonparametric independent continuous variables), or a Student’s t-test (for continuous normally distributed variables). Multivariate regression tools were used in the GLI prediction equations and standard regression checking methods were employed to check model fit, including a review of the residual scatterplots. Logistic regression models were fitted to investigate the risk factors associated with abnormal lung function, covariates in these models were selected based on their statistical significance with a p-value of less than 0.05. Regression results were stratified by study site and the effect sizes were checked to see if there were notable differences by site. Site specific regression results are included in Appendix D5.

A socio-economic (SES) score was calculated from the following inputs: home type, current employment, educational achievement, current income and household assets, Appendix D2. The SES index was categorised and summarized into a binary variable corresponding to lowest versus highest SES.

The use of race in epidemiological studies is contentious [39]; often there are more appropriate underlying covariates which should be used in casual thinking rather than race. However race is widely used to predict spirometry results. To match the methodology used by the Global Lung Initiative, participants’ self-reported race is included in this analysis as a potential predictor of lung function.

2.4 Model Checking of GLI reference equations

In line with the methodology used by the GLI [1], mothers who self-reported that they smoke or who said they had been diagnosed with asthma were dropped from the sample before running the GLI prediction equations and checking the model fit, Appendix D4. The mothers making up this group approximate a group of healthy adults for which the reference equations would provide a good fit if they are appropriate for use in this cohort. A thorough analysis of the predicted values and residuals for the equations indicated that the GLI reference equations do provide a good fit for this cohort of women, suggesting that they are
appropriate for use in a South African context for both black and mixed race women. Appendix D4 provides a detailed description of the analysis of the model residuals.

2.5 Abnormal Lung Function

Abnormal lung function was defined in three different ways; the first was defined by those participants whose observed lung function was outside of the 95% normal limits for them as defined by their GLI reference equations. The second was based on participants who displayed lung function reversibility, and the third was based on the GOLD Spirometric Classification of COPD Severity using the post-bronchodilator FEV$_1$ and FEV$_1$/FVC values. No participants met the requirements in the GOLD classification for COPD, thus this model was not explored further.

3. Results

During the study period, 462 mothers (n=246 (53%) from TC Newman and n=216 (47%) from Mbekweni) were enrolled in the study and performed spirometry.

3.1 Maternal clinical, demographic and socio-economic characteristics

The mean age of mothers was 27 years (sd 5.9, age range 18 – 42 years) and mean BMI 26.4 (sd 5.8), Table C1.

Table C1: Anthropometric, demographic and socioeconomic characteristics

<table>
<thead>
<tr>
<th></th>
<th>Mbekweni</th>
<th>TC Newman</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 216</td>
<td>N = 246</td>
<td>N = 462</td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>Mean (sd)</td>
<td>Mean (sd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>27.8 (6.1)</td>
<td>26.3 (5.6)</td>
<td>27.0 (5.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Height</td>
<td>160.8 (6.8)</td>
<td>158.2 (6.8)</td>
<td>159.4 (6.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Weight</td>
<td>72.1 (16.5)</td>
<td>63.1 (15.0)</td>
<td>67.3 (16.3)</td>
<td>0.00</td>
</tr>
<tr>
<td>BMI</td>
<td>27.8 (5.9)</td>
<td>25.2 (5.5)</td>
<td>26.4 (5.8)</td>
<td>0.00</td>
</tr>
<tr>
<td>Maternal Socio Economic Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest SES</td>
<td>83 (38)</td>
<td>135 (55)</td>
<td>218 (47)</td>
<td>0.00</td>
</tr>
<tr>
<td>Highest SES</td>
<td>133 (62)</td>
<td>111 (45)</td>
<td>244 (53)</td>
<td>0.00</td>
</tr>
</tbody>
</table>
## Ethnicity

<table>
<thead>
<tr>
<th>Ethnicity – Black African</th>
<th>215 (100)</th>
<th>2 (0.8)</th>
<th>217 (47)</th>
<th>0.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous TB disease</td>
<td>8 (4)</td>
<td>10 (4)</td>
<td>18 (4)</td>
<td>0.84</td>
</tr>
<tr>
<td>Pneumonia Admission last 12 months</td>
<td>0 (0)</td>
<td>1 (0.4)</td>
<td>1 (0.2)</td>
<td>0.42</td>
</tr>
<tr>
<td>Bronchitis Admission last 12 months</td>
<td>0 (0)</td>
<td>1 (0.4)</td>
<td>1 (0.2)</td>
<td>0.42</td>
</tr>
<tr>
<td>Emphysema (self-reported history of diagnosis)</td>
<td>1 (0.4)</td>
<td>0 (0)</td>
<td>1 (0.2)</td>
<td>0.28</td>
</tr>
<tr>
<td>Wheeze (self-reported history of diagnosis)</td>
<td>5 (2)</td>
<td>7 (3)</td>
<td>12 (3)</td>
<td>0.72</td>
</tr>
<tr>
<td>Asthma (self-reported history of diagnosis)</td>
<td>5 (2)</td>
<td>6 (3)</td>
<td>11 (2)</td>
<td>0.93</td>
</tr>
<tr>
<td>Current cough (self-reported)</td>
<td>10 (5)</td>
<td>26 (11)</td>
<td>36 (8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Hay fever (self-reported)</td>
<td>8 (4)</td>
<td>17 (7)</td>
<td>25 (6)</td>
<td>0.13</td>
</tr>
<tr>
<td>HIV positive</td>
<td>68 (33)</td>
<td>7 (3)</td>
<td>75 (17)</td>
<td>0.00</td>
</tr>
<tr>
<td>CD4 Median (IQR)</td>
<td>411 (322–659)</td>
<td>419 (334–577)</td>
<td>411 (322–630)</td>
<td>0.79</td>
</tr>
<tr>
<td>Currently taking ARVs</td>
<td>64 (94)</td>
<td>4 (57)</td>
<td>68 (91)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

### SES – Socio Economic Status, TB – tuberculosis, HIV – human immunodeficiency virus, ARV – antiretroviral therapy

Mothers from Mbekweni had a higher BMI compared to mothers at TC Newman (BMI 27.8 vs 25.2, p<0.001). HIV infection (17%) was substantially higher at Mbekweni (33% positive) compared to 3% at TC Newman (p<0.001). Rates of maternal smoking were much higher at TC Newman where 50% of moms were classified as active smokers (compared to 15% at Mbekweni, p=0.05) and 39% classified as passive smoke exposed (50% at Mbekweni, p<0.001). Many of the women in the cohort are pregnant for the first time (36%) with a median parity of 1 previous birth for women in the cohort.

### 3.2 Spirometry Results

The median values before administration of a bronchodilator were FVC (3.11 mL), FEV₁ (2.67 mL) and FEV₁/FVC (87%). After bronchodilator therapy the median values were FVC (3.10 mL), FEV₁ (2.78 mL) and FEV₁/FVC (89%). There were 22 (5%) mothers who had reversible FEV₁, Table C2, of which 7 mothers had partial reversibility and 15 had complete reversibility (which is suggestive of asthma).
Table C2: Bronchodilator Spirometry Results

<table>
<thead>
<tr>
<th></th>
<th>Pre-Bronchodilator Median (IQR)</th>
<th>Post-Bronchodilator Median (IQR)</th>
<th>Difference Median (IQR)</th>
<th>N(%) with 12% or greater difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC (mL)</td>
<td>3.11 (2.84 - 3.42)</td>
<td>3.10 (2.81 - 3.46)</td>
<td>0.00 (-0.05 – 0.07)</td>
<td>n/a *</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; (mL)</td>
<td>2.67 (2.46 - 2.94)</td>
<td>2.78 (2.52 - 3.04)</td>
<td>0.08 (0.02 – 0.14)</td>
<td>22 (5)</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC (%)</td>
<td>86.38 (82.83 - 89.79)</td>
<td>88.62 (85.57 - 91.05)</td>
<td>2.22 (0.60 – 3.65)</td>
<td>n/a *</td>
</tr>
</tbody>
</table>

* 12% of greater difference is only relevant for FEV<sub>1</sub> and thus not calculated for FVC or FEV<sub>1</sub>/FVC

Of the 22 (5%) participants with reversible FEV<sub>1</sub> abnormality only 3 (14%) self-reported that they suffered from asthma. Of the 11 (2.4%) participants who self-reported that they suffered from asthma, 8 (72%) of them did not show any reversibility in their lung function test. Site differences were checked and there was no difference in the rates of reversibility between the two study sites, Appendix D5. There were 19 (4%) mothers who had an unsuccessful test for FVC, FEV<sub>1</sub> or FEV<sub>1</sub>/FVC. No significant differences or trends were noted for those mothers that had an unsuccessful test result; further information about these mothers is included in Appendix D3.
3.3 The Global Lung Initiative’s Reference Equations

The GLI reference equations were used to investigate the prevalence of abnormal lung function within this cohort of women. The following table compares the observed values for FVC, FEV₁ and FEV₁/FVC to the lower and upper limits based on the GLI reference equations. The graphs show the observed spirometry values and the fitted reference equations lines with a 95% CI around the fitted values.

**Table C3: Spirometry values above and below normal limits as defined by GLI reference equations**

<table>
<thead>
<tr>
<th></th>
<th>Values below GLI</th>
<th>Values above GLI</th>
<th>Standardised Z scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower Limit</td>
<td>Upper Limit</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>FEV₁</td>
<td>44 (9.7)</td>
<td>20 (4.4)</td>
<td>-0.3 (-0.9 – 0.4)</td>
</tr>
<tr>
<td>FVC</td>
<td>36 (7.9)</td>
<td>24 (5.3)</td>
<td>-0.2 (-0.9 – 0.5)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>23 (5.1)</td>
<td>12 (2.6)</td>
<td>-0.1 (-0.6 – 0.4)</td>
</tr>
</tbody>
</table>

There were 44 women (10%) who had an observed FEV₁ value that was below the Lower Limit of Normal (LLN) and 20 (4%) who had a value that was above the Upper Limit of Normal (ULN) for FEV₁.
There were 36 women (8%) who had an observed value for FVC below the LLN and 24 (5%) who had a value above the ULN. The results for FEV₁/FVC show that 23 (5%) women had a value below the LLN and 12 women (3%) above the ULN. Overall there were 105 (23%) mothers who had abnormal lung function. Overall the standardized z scores had median values of FEV₁ -0.3, FVC -0.2 and FEV₁/FVC - 0.1.
3.4 Risk Factors associated with respiratory illness

3.4.1 Risk factors associated with abnormal lung function

The univariate and multivariate results for risk factors associated with abnormal lung function are shown in the table below.

Table C4: Univariate and multivariate regression results for abnormal lung function

<table>
<thead>
<tr>
<th></th>
<th>Abnormal FVC</th>
<th></th>
<th>Abnormal FEV₁</th>
<th></th>
<th>Abnormal FEV/FVC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio</td>
<td>95% CI</td>
<td>p-value</td>
<td>Odds Ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.04</td>
<td>0.99 - 1.08</td>
<td>0.13</td>
<td>1.05</td>
<td>1.00 - 1.10</td>
</tr>
<tr>
<td>BMI</td>
<td>1.25</td>
<td>1.05 - 1.33</td>
<td>0.15</td>
<td>1.03</td>
<td>0.98 - 1.10</td>
</tr>
<tr>
<td>Highest SES</td>
<td>0.81</td>
<td>0.47 - 1.40</td>
<td>0.46</td>
<td>0.66</td>
<td>0.39 - 1.12</td>
</tr>
<tr>
<td>Maternal Atopy</td>
<td>1.03</td>
<td>0.58 - 1.83</td>
<td>0.91</td>
<td>0.86</td>
<td>0.49 - 1.50</td>
</tr>
<tr>
<td>Smoking - Nonsmoker</td>
<td>1.00</td>
<td>n/a</td>
<td>n/a</td>
<td>1.00</td>
<td>n/a</td>
</tr>
<tr>
<td>Smoking - Passive</td>
<td>0.64</td>
<td>0.31 - 1.32</td>
<td>0.23</td>
<td>0.43</td>
<td>0.21 - 0.88</td>
</tr>
<tr>
<td>Smoking - Active</td>
<td>0.97</td>
<td>0.48 - 1.98</td>
<td>0.93</td>
<td>0.95</td>
<td>0.49 - 1.85</td>
</tr>
<tr>
<td>HIV</td>
<td>0.89</td>
<td>0.42 - 1.90</td>
<td>0.77</td>
<td>1.07</td>
<td>0.53 - 2.17</td>
</tr>
<tr>
<td>Cough (Any)</td>
<td>1.09</td>
<td>0.41 - 2.92</td>
<td>0.87</td>
<td>3.33</td>
<td>1.20 - 9.21</td>
</tr>
<tr>
<td>TB</td>
<td>1.36</td>
<td>0.38 - 4.84</td>
<td>0.64</td>
<td>2.40</td>
<td>0.62 - 9.29</td>
</tr>
</tbody>
</table>

**MULTIVARIATE ANALYSIS**

<table>
<thead>
<tr>
<th></th>
<th>Abnormal FVC</th>
<th>Abnormal FEV₁</th>
<th>Abnormal FEV/FVC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio</td>
<td>95% CI</td>
<td>p-value</td>
</tr>
<tr>
<td>Age</td>
<td>1.04</td>
<td>0.99 - 1.10</td>
<td>0.08</td>
</tr>
<tr>
<td>BMI</td>
<td>1.40</td>
<td>1.01 - 1.65</td>
<td>0.08</td>
</tr>
<tr>
<td>Highest SES</td>
<td>n/a*</td>
<td>n/a*</td>
<td>n/a*</td>
</tr>
<tr>
<td>TB</td>
<td>n/a*</td>
<td>n/a*</td>
<td>n/a*</td>
</tr>
</tbody>
</table>

*SES – Socio Economic Status, TB – tuberculosis, HIV – human immunodeficiency virus, BMI – body mass index

*only significant covariates included in the multivariate model, SES and TB excluded from multivariate FVC and FEV1/FVC model as they were non-significant at the 10% level.

From the results, a high BMI is associated with a higher risk for poor FVC with an odds ratio of 1.40 (CI: 1.01, 1.65). High BMI is also shown to be associated with an abnormal FEV/FVC result with an OR 1.25 (CI 1.10, 1.95).
SES and TB were only significantly associated with an abnormal FEV\textsubscript{1} result. The results show there is some evidence to suggest that mothers with a higher SES have better FEV\textsubscript{1} results than those mothers with a lower SES score, with the adjusted SES OR 0.65 (CI 0.36 ,1.08). TB has an odds ratio of 3.06 (1.07; 8.74), those mothers who self-reported that they had previously had TB had a 3.06 higher odds of having an abnormal FEV\textsubscript{1} result.

### 3.4.2 Risk factors associated with reversible obstructive airflow disease

The univariate and multivariate results for risk factors associated with reversible FEV\textsubscript{1} are shown in the table below.

<table>
<thead>
<tr>
<th>ReversibleFEV\textsubscript{1}</th>
<th>UNIVARIATE ANALYSIS</th>
<th>MULTIVARIATE MODEL</th>
<th>Pseudo R\textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio</td>
<td>95% CI</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.99</td>
<td>0.92</td>
<td>1.07</td>
</tr>
<tr>
<td>BMI</td>
<td>0.97</td>
<td>0.90</td>
<td>1.05</td>
</tr>
<tr>
<td>Highest SES*</td>
<td>1.08</td>
<td>0.46</td>
<td>2.54</td>
</tr>
<tr>
<td>Maternal Atopy</td>
<td>3.48</td>
<td>1.38</td>
<td>8.81</td>
</tr>
<tr>
<td>Smoking – Passive*</td>
<td>1.02</td>
<td>0.34</td>
<td>3.08</td>
</tr>
<tr>
<td>Smoking – Active*</td>
<td>0.79</td>
<td>0.23</td>
<td>2.66</td>
</tr>
<tr>
<td>HIV*</td>
<td>1.12</td>
<td>0.37</td>
<td>3.42</td>
</tr>
<tr>
<td>Cough (Any)*</td>
<td>0.55</td>
<td>0.07</td>
<td>4.22</td>
</tr>
<tr>
<td>TB*</td>
<td>2.65</td>
<td>0.57</td>
<td>12.32</td>
</tr>
<tr>
<td>AsthmaAny</td>
<td>8.53</td>
<td>2.09</td>
<td>34.72</td>
</tr>
</tbody>
</table>

* excluded from the multivariate model as covariate was non-significant at the 10% level

Maternal atopy is strongly associated with reversible FEV\textsubscript{1} with an odds ratio of 3.42 (CI 1.29, 9.70), self-reported asthma is also strongly associated with reversibility in FEV\textsubscript{1} with an odds ratio of 6.63 (CI 1.24, 32.14).
5. Discussion

The results of this study showed that while only a small proportion (5%) of young adult women in this setting had reversible FEV₁, the vast majority of these (86%) did not self-report a previous diagnosis with asthma or another lung disease. Of those with reversible FEV₁, most (68%) had completely reversible lung function. A small but appreciable proportion of participants had abnormal values for FEV₁/FVC and/or FEV₁/FVC ratio, but no participants had spirometry results that classified them as having COPD according to the GOLD Spirometric Classification. In addition, the GLI reference equations performed well at predicting spirometry results for this cohort of women.

Previous studies have suggested that the global prevalence of doctor diagnosed asthma and clinical/treated asthma are both approximately 4-5% [15]. In South Africa the prevalence of asthma has been estimated to be approximately 8% across all ages [12]. The BOLD study estimated the prevalence of stage I COPD to be 5% of the South African population, stage II was estimated to be 12% and stage III or higher was 7% of the population [2]. The FRESH AIR study which also classified participants according to spirometry results noted that 6.7% of women in the study aged 30 – 39 years could be classified as having COPD and 2% of the entire study population had asthma [8]. Compared to these prevalence rates it would seem that the rates of abnormal lung function in the Drakenstein cohort are fairly high.

Of those mothers who had reversible FEV₁, 86% (19/22) of them did not report a previous diagnosis of asthma or COPD, suggesting undiagnosed lung disease. This prevalence of undiagnosed reversibility corresponds to other studies which have suggested that self-reported rates of lung disease are often an unreliable guide to the true prevalence of disease [11]. Given the rates of undiagnosed reversibility it is likely that the true prevalence of lung disease in South Africa is likely to be much higher than current estimates. Maternal atopy was shown to be strongly associated with reversibility (odds ratio 3.54, CI 1.29, 9.70) and is well known to be associated with asthma. There was a large percentage of mothers in this cohort who were classified as being atopic (n=168, 38%), highlighting the need for more widely available spirometry testing in young adults in South Africa.
A review of the model fit for the Global Lung Initiatives reference equations showed that the reference equations were able to predict lung function remarkably well in this group of young, healthy women. This suggests that the GLI reference equations are suitable for use in this cohort of South African adult females (although further research is needed to determine the suitability for males). This finding is especially useful given the need for spirometry reference equations in South Africa. Another African study, the FRESH AIR study, also found the GLI reference equations appropriate for their cohort of adult men and women in Uganda [8]. Currently, the Global Lung Initiative does not use data from sub-Saharan Africa in its work, but plans to update the sample used for the reference equations to include a larger sample from African countries. Preliminary findings suggest that the equations are already very useful in an African context and further improvements should lead to an even better model fit for individuals in Africa. This will be a notable achievement for public health and will significantly improve lung disease screening, diagnosis and treatment rates in African countries.

This study examines lung health in younger adults and highlights the prevalence of abnormal lung function in this group. There have been few studies which explore lung disease in younger adults in sub-Saharan Africa, as previous literature and recent studies on lung disease in Africa have focused on the prevalence of COPD and Asthma in older populations [2] [7] [8]. This study makes an important contribution by demonstrating that an appreciable proportion of women of reproductive age in this setting are affected by abnormal lung function which is not specifically asthma or COPD.

Several limitations should be considered when reviewing these findings. Although there were high rates of HIV in this cohort, this study did not show any significant associations between HIV and lung function. It has been suggested that spirometry has limited sensitivity for early HIV-associated lung disease; lung diffusion has been proposed as a better lung function measurement tool in this area [23]. Further, almost all HIV-infected women in this cohort were on ART and relatively healthy. Participants in this study were mothers who were 6-10 weeks post-partum, and research suggests that lung function returns to normal within this time post pregnancy [42], but there are a number of socio-economic and
post-pregnancy health related factors that could influence overall health and lung function. Lastly, lung function reversibility was defined as a significant improvement in FEV$_1$ after bronchodilator, other studies have suggested that one should consider both FEV$_1$ and FVC when defining reversibility [6].

Given the high prevalence of smoking in this population, it was surprising that the risk factor analysis did not show a strong association between smoking and abnormal lung function. It is possible that there were too few non-smokers in this cohort to detect a statistically significant result between groups, only 119 (26%) of mothers were not classified as smokers or passive smoke exposed. Additionally smoking duration is relatively short and the effects of it probably not yet seen in this cohort.

This study had a number of strengths. Well-designed studies collecting spirometry data in African settings are scarce. Little is known about adult lung function in peri-urban communities in South Africa, and this sample size here of 462 allowed power to detect appreciable associations in this context. The lung function tests were done by experienced staff using equipment that was calibrated correctly and the collected data was continually checked for quality control errors. The study made use of both self-reported data and a number of robust clinical measures for weight, height, HIV, smoking and maternal atopy. Future research should build on the evidence that suggests the GLI reference equations are appropriate for use in this cohort, and test the equations in women, men and children of various ages. Additional studies should be conducted to further investigate the prevalence of abnormal lung function in South African communities.

In conclusion, this study makes a useful contribution to the body of knowledge about lung function in adult South African females and the use of the GLI reference equations in South Africa. The findings confirm the importance of spirometry testing for young adults, and strengthen the evidence that undiagnosed asthma and abnormal lung function should be a public health concern in South Africa.
References


D. APPENDICES
Appendix D1: Ethics approval form

UCT HREC 913 / 2014

26 January 2015

HREC REF: 913/2014

Prof L Myer
Public Health & Family Medicine
Falmouth Building

Dear Prof Myer

PROJECT TITLE: NORMAL VALUES AND PREDICTORS OF LUNG FUNCTION IN HEALTHY SOUTH AFRICAN FEMALES: A STUDY TO DETERMINE AVERAGE LUNG INFECTION REFERENCE DATA IN A COHORT OF HEALTHY SOUTH AFRICAN WOMEN, WITH A REVIEW OF CURRENTLY USED REFERENCE EQUATIONS AND INVESTIGATION INTO PREDICTORS OF LOW LUNG FUNCTION -linked to 401/2009 (Master candidate-Emilee Smith)

Thank you for your response letter to the Faculty of Health Sciences Human Research Ethics Committee dated 14 January 2015.

It is a pleasure to inform you that the HREC has formally approved the above-mentioned study.

Approval is granted for one year until the 30th January 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the student, Emilee Smith will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWAD00001637,
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

HREC 913/2014
Appendix D2: Calculation of SES score

A Socio-Economic (SES) score was used in the analysis, this score was calculated with the following inputs:

- home type, assigned a value of “1” for house / flat or “0” for shack / informal home
- current employment, assigned a value of “1” for working or “0” for not working
- educational achievement, assigned a value of “1” for primary education achieved, a “2” for some secondary, a “3” for completed secondary, and a “4” for any tertiary
- income category, assigned a value of “1” for < R1,000 per month, “2” for R1,000 - R5,000 / m, “3” for > R5,000 pm
- Household assets, a score of “1” for any of the following: electricity, running water, flush toilet, kitchen sink, electric stove, working telephone/ cell phone, a motor car, motorcycle / scooter, bicycle.

A weighted average SES score was calculated which adds up the standardized values of each of the above inputs. From this the SES score was categorized into quartiles to form the four groups of lowest SES, low-mod SES, mod-high SES and highest SES.

The SES index was categorised into quartiles to form four groups which were lowest SES, low-mod SES, mod-high SES and highest SES. For some of the spirometry outcomes there were very few participants in category 3 “mod-high SES” which made model fitting with 4 categories difficult, thus the categories were summarized into a binary variable with “0” for lowest SES and low-mod SES and “1” for mod-high SES and highest SES.
In order to review the psychometric properties of this measure the item-item associations were reviewed with box plots for categorical measures and a scatter plot for the continuous measure asset sum. This was done to review whether the constructed SES score was distributed equally across the selected input variables. An assessment of the following graphs indicated that the constructed SES score is indeed distributed equally over the independent input factors.

Figure D1: Box plot of the SES score by Employment Status

Figure D2: Box plot of the SES score by Income Category
**Figure D3:** Box plot of the SES score by Home Type

**Figure D4:** Box plot of the SES score by Education
Figure D5: Scatter plot of the SES score by Asset Sum
Appendix D3: Mothers with unsuccessful lung function test results

The majority of mothers had successful tests on all of the measures but some had poor results for FVC, FEV$_1$ or FEV$_1$/FVC. There were 19 (4%) mothers who had an unsuccessful test for FVC, FEV$_1$ or FEV$_1$/FVC.

The table below shows the number of unsuccessful test results for each spirometry measure.

### Table D1: The number of unsuccessful test results for each spirometry measure

<table>
<thead>
<tr>
<th></th>
<th>FVC N(%)</th>
<th>FEV$_1$ N(%)</th>
<th>FEV1/FVC N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Bronchodilator</td>
<td>0 (0)</td>
<td>2 (0.4)</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Post-Bronchodilator</td>
<td>6 (1.3)</td>
<td>11 (2.3)</td>
<td>17 (3.6)</td>
</tr>
</tbody>
</table>

### Table D2: Review of mother’s with unsuccessful lung function tests

<table>
<thead>
<tr>
<th></th>
<th>Successful Test</th>
<th>Unsuccessful Test</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 443</td>
<td>N = 19</td>
<td>N =462</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (sd)</td>
<td>Mean (sd)</td>
<td>Mean (sd)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>27 (6)</td>
<td>27 (6)</td>
<td>27 (6)</td>
<td>0.71</td>
</tr>
<tr>
<td>Height</td>
<td>159 (7)</td>
<td>160 (6)</td>
<td>159 (7)</td>
<td>0.63</td>
</tr>
<tr>
<td>Weight</td>
<td>67 (16)</td>
<td>70 (18)</td>
<td>67 (16)</td>
<td>0.81</td>
</tr>
<tr>
<td>BMI</td>
<td>26 (6)</td>
<td>27 (7)</td>
<td>26 (6)</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>N(%)</td>
<td>N(%)</td>
<td>N(%)</td>
<td></td>
</tr>
<tr>
<td>Maternal SES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest SES - Low-moderate SES</td>
<td>206 (47)</td>
<td>12 (63)</td>
<td>218 (47)</td>
<td>0.15</td>
</tr>
<tr>
<td>Mod-High SES - High SES</td>
<td>237 (53)</td>
<td>7 (37)</td>
<td>244 (53)</td>
<td>0.14</td>
</tr>
<tr>
<td>Ethnicity – Black African</td>
<td>209 (47)</td>
<td>8 (42)</td>
<td>217 (47)</td>
<td>0.66</td>
</tr>
<tr>
<td>Previous TB infection</td>
<td>18 (4)</td>
<td>0 (0)</td>
<td>18 (4)</td>
<td>0.37</td>
</tr>
<tr>
<td>Pneumonia Admission last 12 months</td>
<td>1 (0.2)</td>
<td>0 (0)</td>
<td>1(0.2)</td>
<td>0.89</td>
</tr>
<tr>
<td>Bronchitis Admission last 12 months</td>
<td>1 (0.2)</td>
<td>0(0)</td>
<td>1 (0.2)</td>
<td>0.89</td>
</tr>
<tr>
<td>Emphysema (self-reported history of diagnosis)</td>
<td>1 (0.2)</td>
<td>0(0)</td>
<td>1 (0.2)</td>
<td>0.82</td>
</tr>
<tr>
<td>Wheeze (self-reported history of diagnosis)</td>
<td>11 (3)</td>
<td>1 (5)</td>
<td>12 (3)</td>
<td>0.46</td>
</tr>
<tr>
<td>Asthma (self-reported history of diagnosis)</td>
<td>10 (2)</td>
<td>1 (5)</td>
<td>11 (2)</td>
<td>0.40</td>
</tr>
<tr>
<td>Current cough (self-reported)</td>
<td>34 (8)</td>
<td>2 (11)</td>
<td>36 (8)</td>
<td>0.65</td>
</tr>
<tr>
<td>Hay fever (self-reported)</td>
<td>21 (5)</td>
<td>4 (21)</td>
<td>25 (5)</td>
<td>0.002</td>
</tr>
<tr>
<td>HIV positive</td>
<td>73 (17)</td>
<td>2 (11)</td>
<td>75 (17)</td>
<td>0.46</td>
</tr>
<tr>
<td>CD4 Median (IQR)</td>
<td>416 (322 – 630)</td>
<td>241 (241 – 241)</td>
<td>411 (322 – 630)</td>
<td>0.18</td>
</tr>
<tr>
<td>Currently taking ARVs</td>
<td>66 (15)</td>
<td>2 (11)</td>
<td>68 (15)</td>
<td>0.60</td>
</tr>
<tr>
<td>Smoking (Urine Cotinine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Active smoker</td>
<td>144 (34)</td>
<td>5 (28)</td>
<td>149 (34)</td>
<td>0.57</td>
</tr>
<tr>
<td>Passive smoker</td>
<td>185 (44)</td>
<td>9 (50)</td>
<td>194 (44)</td>
<td>0.63</td>
</tr>
<tr>
<td>Maternal Atopy</td>
<td>159 (38)</td>
<td>9 (47)</td>
<td>168 (38)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

No significant differences or trends were noted for those mothers that had an unsuccessful test result.
Appendix D4: GLI Model Checking

In line with the methodology used by the Global Lung Initiative [1], mothers who self-reported that they smoke or who said they suffered from asthma were dropped from the sample before running the GLI prediction equations and checking the model fit. There were 117 (26%) mothers who self-reported that they currently smoke and 11 (2%) who self-reported that they suffered from asthma. After dropping these mothers there were 337 adult females left in the sample to check the GLI reference equations model fit.

The mothers making up this group approximate a group of healthy adults for which the reference equations would provide a good fit if they are appropriate for use in this cohort. A thorough analysis of the predicted values and residuals for the equations indicate that the GLI reference equations do provide a good fit for this cohort of women. The histograms below show the distribution of the model residuals, this can be used to review the assumption of normality. All of the residual distributions look approximately normal.

Figure D6: Histograms of model residuals for FVC GLI reference equation
Figure D7: Histograms of model residuals for FVC GLI reference equation

Figure D8: Histograms of model residuals for FVC GLI reference equation
Table 8 below shows the mean observed values for FEV\textsubscript{1}, FVC and FEV\textsubscript{1}/FVC, the predicted values, and the residuals. The mean values for all residuals are close to zero.

<table>
<thead>
<tr>
<th>FEV</th>
<th>mean (sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV observed</td>
<td>2.72 (0.44)</td>
</tr>
<tr>
<td>FEV Predicted</td>
<td>2.80 (0.27)</td>
</tr>
<tr>
<td>Residuals</td>
<td>-0.07 (0.44)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FVC</th>
<th>mean (sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC observed</td>
<td>3.15 (0.50)</td>
</tr>
<tr>
<td>FVC Predicted</td>
<td>3.23 (0.32)</td>
</tr>
<tr>
<td>Residuals</td>
<td>-0.08 (0.47)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FEV/FVC</th>
<th>mean (sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV/FVC observed</td>
<td>0.86 (0.06)</td>
</tr>
<tr>
<td>FEV/FVC Predicted</td>
<td>0.87 (0.02)</td>
</tr>
<tr>
<td>Residuals</td>
<td>-0.01 (0.06)</td>
</tr>
</tbody>
</table>

The diagrams below show a scatter plot between the predicted values and the model residuals. This provides a test of heteroscedasticity. It is a requirement of a linear regression models that there is not substantial variation within the variance term. The graphs below show no linear trend or heteroscedasticity. A review of these graphs indicates that the variability is approximately equal across all values of the independent variable for each model.

Figure D9: Scatter plot of residuals: FEV\textsubscript{1}
Figure D10: Scatter plot of residuals: FVC

Figure D11: Scatter plot of residuals: FEV/FVC
Appendix D5: Site Stratified Spirometry Results and Regression Models

The tables below show the spirometry data and results of the multivariate regression analysis stratified by study site. The stratified analysis was done to determine whether there were any notable differences between effects sizes at the two different sites. Measurements and effects were similar across the two different sites.

Table D4: Pre-Bronchodilator FVC, FEV\textsubscript{1} and FEV\textsubscript{1}/FVC results by study site

<table>
<thead>
<tr>
<th></th>
<th>Mbekweni Median (IQR)</th>
<th>TC Newman Median (IQR)</th>
<th>Total Median (IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC (L)</td>
<td>3.15 (2.87 – 3.46)</td>
<td>3.08 (2.81 – 3.40)</td>
<td>3.11 (2.84 – 3.42)</td>
<td>0.23</td>
</tr>
<tr>
<td>FEV\textsubscript{1} (L)</td>
<td>2.70 (2.45 – 2.96)</td>
<td>2.65 (2.46 – 2.93)</td>
<td>2.67 (2.46 – 2.94)</td>
<td>0.49</td>
</tr>
<tr>
<td>FEV\textsubscript{1}/FVC (%)</td>
<td>85.89 (82.63 – 89.03)</td>
<td>87.01 (83.41 – 90.17)</td>
<td>86.38 (82.83 – 89.79)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table D5: Post-Bronchodilator FVC, FEV\textsubscript{1} and FEV\textsubscript{1}/FVC results by study site

<table>
<thead>
<tr>
<th></th>
<th>Mbekweni Median (IQR)</th>
<th>TC Newman Median (IQR)</th>
<th>Total Median (IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC (L)</td>
<td>3.16 (2.86 – 3.50)</td>
<td>3.08 (2.79 – 3.40)</td>
<td>3.10 (2.81 – 3.46)</td>
<td>0.18</td>
</tr>
<tr>
<td>FEV\textsubscript{1} (L)</td>
<td>2.78 (2.54 – 3.06)</td>
<td>2.77 (2.52 – 3.02)</td>
<td>2.78 (2.52 – 3.04)</td>
<td>0.47</td>
</tr>
<tr>
<td>FEV\textsubscript{1}/FVC (%)</td>
<td>87.80 (85.34 – 90.56)</td>
<td>88.28 (85.91 – 91.87)</td>
<td>88.62 (85.57 – 91.05)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Table D6: Levels of reversibility in FEV\textsubscript{1} results by study site

<table>
<thead>
<tr>
<th></th>
<th>Mbekweni N (%)</th>
<th>TC Newman N (%)</th>
<th>Total N (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No reversibility</td>
<td>206 (95)</td>
<td>234 (95)</td>
<td>440 (95)</td>
<td>1.00</td>
</tr>
<tr>
<td>Reversibility</td>
<td>10 (5)</td>
<td>12 (5)</td>
<td>22 (5)</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Table D7: Multivariate regression results stratified by study site

<table>
<thead>
<tr>
<th></th>
<th>Mbekweni</th>
<th>TC Newman</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abnormal FVC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pseudo R²</strong></td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>Age</td>
<td>1.04</td>
<td>1.05</td>
</tr>
<tr>
<td>BMI</td>
<td>1.03</td>
<td>0.94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mbekweni</th>
<th>TC Newman</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abnormal FEV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pseudo R²</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.00</td>
<td>1.07</td>
</tr>
<tr>
<td>BMI</td>
<td>1.05</td>
<td>0.99</td>
</tr>
<tr>
<td>High SES</td>
<td>0.80</td>
<td>0.64</td>
</tr>
<tr>
<td>TB</td>
<td>2.64</td>
<td>3.85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mbekweni</th>
<th>TC Newman</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abnormal FEV/FVC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pseudo R²</strong></td>
<td>21%</td>
<td>9%</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.02</td>
<td>1.00</td>
</tr>
<tr>
<td>BMI</td>
<td>1.02</td>
<td>0.92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mbekweni</th>
<th>TC Newman</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reversible FEV1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pseudo R²</strong></td>
<td>21%</td>
<td>9%</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.94</td>
<td>1.04</td>
</tr>
<tr>
<td>BMI</td>
<td>0.97</td>
<td>1.02</td>
</tr>
<tr>
<td>Maternal Atopy</td>
<td>11.08</td>
<td>2.55</td>
</tr>
<tr>
<td>AsthmaAny</td>
<td>3.24</td>
<td>14.64</td>
</tr>
</tbody>
</table>
Appendix D6: Journal Guidelines for Authors, Respiratory Research

Instructions for authors

Research Articles

Presubmission enquiries | Submission process | Preparing main manuscript text | Preparing illustrations and figures | Preparing tables | Preparing additional files | Style and language

See 'About this journal' for descriptions of different article types and information about policies and the refereeing process.

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The following word processor file formats are acceptable for the main manuscript document:

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- Rich text format (RTF)
Preparing main manuscript text

General guidelines of the journal's style and language are given below.

Overview of manuscript sections for Research Articles

Manuscripts for Research Articles submitted to *Respiratory Research* should be divided into the following sections (in this order):

- Title page
- Abstract
- Keywords
- Background
- Methods
- Results and discussion
- Conclusions
- List of abbreviations used (if any)
- Competing interests
- Authors' contributions
- Authors' information
- Acknowledgements
- Endnotes
- References
- Illustrations and figures (if any)
- Tables and captions
- Preparing additional files

The Accession Numbers of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript should be provided, in square brackets and include the corresponding database name; for example, [EMBL:AB026295, EMBL:AC137000, DDBJ:AE000812, GenBank:U49845, PDB:1BFM, Swiss-Prot:Q96KQ7, PIR:S66116].

The databases for which we can provide direct links are: EMBL Nucleotide Sequence Database ([EMBL](http://www.ebi.ac.uk/EMBL-EBI/)), DNA Data Bank of Japan ([DDBJ](http://www.ddbj.nig.ac.jp/)), GenBank at the NCBI ([GenBank](http://www.ncbi.nlm.nih.gov/)), Protein Data Bank ([PDB](http://www.pdb.org/)), Protein Information Resource ([PIR](http://www.ncbi.nlm.nih.gov/)) and the Swiss-Prot Protein Database ([Swiss-Prot](http://www.uniprot.org/)).

You can [download a template](http://www.bmc.com/submit/Template/TemplateDownload.aspx) (Mac and Windows compatible; Microsoft Word 98/2000) for your article.

For reporting standards please see the information in the About section.
Title page

The title page should:

- provide the title of the article
- list the full names, institutional addresses and email addresses for all authors
- indicate the corresponding author

Please note:

- the title should include the study design, for example "A versus B in the treatment of C: a randomized controlled trial X is a risk factor for Y: a case control study"
- abbreviations within the title should be avoided

Abstract

The Abstract of the manuscript should not exceed 350 words and must be structured into separate sections: Background, the context and purpose of the study; Methods, how the study was performed and statistical tests used; Results, the main findings; Conclusions, brief summary and potential implications. Please minimize the use of abbreviations and do not cite references in the abstract. Trial registration, if your research reports the results of a controlled health care intervention, please list your trial registry, along with the unique identifying number (e.g. Trial registration: Current Controlled Trials ISRCTN73824458). Please note that there should be no space between the letters and numbers of your trial registration number. We recommend manuscripts that report randomized controlled trials follow the CONSORT extension for abstracts.

Keywords

Three to ten keywords representing the main content of the article.

Background

The Background section should be written in a way that is accessible to researchers without specialist knowledge in that area and must clearly state - and, if helpful, illustrate - the background to the research and its aims. Reports of clinical research should, where appropriate, include a summary of a search of the literature to indicate why this study was necessary and what it aimed to contribute to the field. The section should end with a brief statement of what is being reported in the article.

Methods

The methods section should include the design of the study, the setting, the type of participants or materials involved, a clear description of all interventions and comparisons, and the type of analysis used, including a power calculation if appropriate. Generic drug names should generally be used. When proprietary brands are used in research, include the brand names in parentheses in the Methods section.

For studies involving human participants a statement detailing ethical approval and consent should be included in the methods section. For further details of the journal's editorial policies and ethical guidelines see 'About this journal'.

For further details of the journal's data-release policy, see the policy section in 'About this journal'.

Results and discussion

The Results and discussion may be combined into a single section or presented separately. Results of statistical analysis should include, where appropriate, relative and absolute risks or risk reductions, and confidence intervals. The Results and discussion sections may also be broken into subsections with short, informative headings.

Conclusions
This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations can be provided, which should precede the competing interests and authors' contributions.

Competing interests

A competing interest exists when your interpretation of data or presentation of information may be influenced by your personal or financial relationship with other people or organizations. Authors must disclose any financial competing interests; they should also reveal any non-financial competing interests that may cause them embarrassment were they to become public after the publication of the manuscript.

Authors are required to complete a declaration of competing interests. All competing interests that are declared will be listed at the end of published articles. Where an author gives no competing interests, the listing will read 'The author(s) declare that they have no competing interests'.

When completing your declaration, please consider the following questions:

Financial competing interests

- In the past three years have you received reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? Is such an organization financing this manuscript (including the article-processing charge)? If so, please specify.
- Do you hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? If so, please specify.
- Do you hold or are you currently applying for any patents relating to the content of the manuscript? Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript? If so, please specify.
- Do you have any other financial competing interests? If so, please specify.

Non-financial competing interests

Are there any non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this manuscript? If so, please specify.

If you are unsure as to whether you, or one your co-authors, has a competing interest please discuss it with the editorial office.

Authors' contributions

In order to give appropriate credit to each author of a paper, the individual contributions of authors to the manuscript should be specified in this section.

According to ICMJE guidelines, An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. To qualify as an author one should 1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; 3) have given final approval of the version to be published; and 4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.

We suggest the following kind of format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT...
Emilee Smith MPH Thesis

participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

All contributors who do not meet the criteria for authorship should be listed in an acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support.

Authors' information

You may choose to use this section to include any relevant information about the author(s) that may aid the reader's interpretation of the article, and understand the standpoint of the author(s). This may include details about the authors' qualifications, current positions they hold at institutions or societies, or any other relevant background information. Please refer to authors using their initials. Note this section should not be used to describe any competing interests.

Acknowledgements

Please acknowledge anyone who contributed towards the article by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include the source(s) of funding for each author, and for the manuscript preparation. Authors must describe the role of the funding body, if any, in design, in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. Please also acknowledge anyone who contributed materials essential for the study. If a language editor has made significant revision of the manuscript, we recommend that you acknowledge the editor by name, where possible.

The role of a scientific (medical) writer must be included in the acknowledgements section, including their source(s) of funding. We suggest wording such as 'We thank Jane Doe who provided medical writing services on behalf of XYZ Pharmaceuticals Ltd.'

Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements section.

Endnotes

Endnotes should be designated within the text using a superscript lowercase letter and all notes (along with their corresponding letter) should be included in the Endnotes section. Please format this section in a paragraph rather than a list.

References

All references, including URLs, must be numbered consecutively, in square brackets, in the order in which they are cited in the text, followed by any in tables or legends. Each reference must have an individual reference number. Please avoid excessive referencing. If automatic numbering systems are used, the reference numbers must be finalized and the bibliography must be fully formatted before submission.

Only articles, clinical trial registration records and abstracts that have been published or are in press, or are available through public e-print/preprint servers, may be cited; unpublished abstracts, unpublished data and personal communications should not be included in the reference list, but may be included in the text and referred to as "unpublished observations" or "personal communications" giving the names of the involved researchers. Obtaining permission to quote personal communications and unpublished data from the cited colleagues is the responsibility of the author. Footnotes are not allowed, but endnotes are permitted. Journal abbreviations follow Index Medicus/MEDLINE. Citations in the reference list should include all named authors, up to the first six before adding 'et al.'.

Any in press articles cited within the references and necessary for the reviewers' assessment of the manuscript should be made available if requested by the editorial office.

An Endnote style file is available.

Examples of the Respiratory Research reference style are shown below. Please ensure that the reference style is followed precisely; if the references are not in the correct style they may have to be retyped and carefully proofread.
All web links and URLs, including links to the authors' own websites, should be given a reference number and included in the reference list rather than within the text of the manuscript. They should be provided in full, including both the title of the site and the URL, in the following format: The Mouse Tumor Biology Database [http://tumor.informatics.jax.org/mtbwi/index.do]. If an author or group of authors can clearly be associated with a web link, such as for weblogs, then they should be included in the reference.

Examples of the Respiratory Research reference style

*Article within a journal*


*Article within a journal (no page numbers)*


*Article within a journal by DOI*


*Article within a journal supplement*


*Book chapter, or an article within a book*


*OnlineFirst chapter in a series (without a volume designation but with a DOI)*


*Complete book, authored*


*Online document*


*Online database*


*Supplementary material/private homepage*


*University site*


*FTP site*


*Organization site*


**Preparing illustrations and figures**
Illustrations should be provided as separate files, not embedded in the text file. Each figure should include a single illustration and should fit on a single page in portrait format. If a figure consists of separate parts, it is important that a single composite illustration file be submitted which contains all parts of the figure. There is no charge for the use of color figures.

Please read our figure preparation guidelines for detailed instructions on maximising the quality of your figures.

**Formats**

The following file formats can be accepted:

- PDF (preferred format for diagrams)
- DOCX/DOC (single page only)
- PPTX/PPT (single slide only)
- EPS
- PNG (preferred format for photos or images)
- TIFF
- JPEG
- BMP

**Figure legends**

The legends should be included in the main manuscript text file at the end of the document, rather than being a part of the figure file. For each figure, the following information should be provided: Figure number (in sequence, using Arabic numerals - i.e. Figure 1, 2, 3 etc); short title of figure (maximum 15 words); detailed legend, up to 300 words.

**Preparing a personal cover page**

If you wish to do so, you may submit an image which, in the event of publication, will be used to create a cover page for the PDF version of your article. The cover page will also display the journal logo, article title and citation details. The image may either be a figure from your manuscript or another relevant image. You must have permission from the copyright to reproduce the image. Images that do not meet our requirements will not be used.

Images must be 300dpi and 155mm square (1831 x 1831 pixels for a raster image).

Allowable formats - EPS, PDF (for line drawings), PNG, TIFF (for photographs and screen dumps), JPEG, BMP, DOC, PPT, CDX, TGF (ISIS/Draw).

**Preparing tables**

Each table should be numbered and cited in sequence using Arabic numerals (i.e. Table 1, 2, 3 etc.). Tables should also have a title (above the table) that summarizes the whole table; it should be no longer than 15 words. Detailed legends may then follow, but they should be concise. Tables should always be cited in text in consecutive numerical order.

Smaller tables considered to be integral to the manuscript can be pasted into the end of the document text file, in A4 portrait or landscape format. These will be typeset and displayed in the final published form of the article. Such tables should be formatted using the 'Table object' in a word processing program to ensure that columns of data are kept aligned when the file is sent electronically for review; this will not always be the case if columns are generated by simply using tabs to separate text. Columns and rows of data should be made visibly distinct by ensuring that the borders of each cell display as black lines. Commas should not be used to indicate numerical values. Color and shading may not be used; parts of the table can be highlighted using symbols or bold text, the meaning of which should be explained in a table legend. Tables should not be embedded as figures or spreadsheet files.
Larger datasets or tables too wide for a landscape page can be uploaded separately as additional files. Additional files will not be displayed in the final, laid-out PDF of the article, but a link will be provided to the files as supplied by the author.

Tabular data provided as additional files can be uploaded as an Excel spreadsheet (.xls) or comma separated values (.csv). As with all files, please use the standard file extensions.

Preparing additional files

Although Respiratory Research does not restrict the length and quantity of data included in an article, we encourage authors to provide datasets, tables, movies, or other information as additional files.

Please note: All Additional files will be published along with the article. Do not include files such as patient consent forms, certificates of language editing, or revised versions of the main manuscript document with tracked changes. Such files should be sent by email to respiratory-research@biomedcentral.com, quoting the Manuscript ID number.

Results that would otherwise be indicated as "data not shown" can and should be included as additional files. Since many weblinks and URLs rapidly become broken, Respiratory Research requires that supporting data are included as additional files, or deposited in a recognized repository. Please do not link to data on a personal/departmental website. The maximum file size for additional files is 20 MB each, and files will be virus-scanned on submission.

Additional files can be in any format, and will be downloadable from the final published article as supplied by the author. We recommend CSV rather than PDF for tabular data.

Certain supported files formats are recognized and can be displayed to the user in the browser. These include most movie formats (for users with the Quicktime plugin), mini-websites prepared according to our guidelines, chemical structure files (MOL, PDB), geographic data files (KML).

If additional material is provided, please list the following information in a separate section of the manuscript text:

- File name (e.g. Additional file 1)
- File format including the correct file extension for example .pdf, .xls, .txt, .pptx (including name and a URL of an appropriate viewer if format is unusual)
- Title of data
- Description of data

Additional files should be named "Additional file 1" and so on and should be referenced explicitly by file name within the body of the article, e.g. 'An additional movie file shows this in more detail [see Additional file 1].'

Additional file formats

Ideally, file formats for additional files should not be platform-specific, and should be viewable using free or widely available tools. The following are examples of suitable formats.

- Additional documentation
  - PDF (Adobe Acrobat)
- Animations
  - SWF (Shockwave Flash)
- Movies
  - MP4 (MPEG 4)
  - MOV (Quicktime)
- Tabular data
  - XLS, XLSX (Excel Spreadsheet)
  - CSV (Comma separated values)

As with figure files, files should be given the standard file extensions.
Mini-websites

Small self-contained websites can be submitted as additional files, in such a way that they will be browsable from within the full text HTML version of the article. In order to do this, please follow these instructions:

1. Create a folder containing a starting file called index.html (or index.htm) in the root.
2. Put all files necessary for viewing the mini-website within the folder, or sub-folders.
3. Ensure that all links are relative (i.e., "images/picture.jpg" rather than "/images/picture.jpg" or "http://yourdomain.net/images/picture.jpg" or "C:\Documents and Settings\username\My Documents\mini-website\images\picture.jpg") and no link is longer than 255 characters.
4. Access the index.html file and browse around the mini-website, to ensure that the most commonly used browsers (Internet Explorer and Firefox) are able to view all parts of the mini-website without problems, it is ideal to check this on a different machine.
5. Compress the folder into a ZIP, check the file size is under 20 MB, ensure that index.html is in the root of the ZIP, and that the file has .zip extension, then submit as an additional file with your article.

Style and language

General

Currently, Respiratory Research can only accept manuscripts written in English. Spelling should be US English or British English, but not a mixture.

There is no explicit limit on the length of articles submitted, but authors are encouraged to be concise.

Respiratory Research will not edit submitted manuscripts for style or language; reviewers may advise rejection of a manuscript if it is compromised by grammatical errors. Authors are advised to write clearly and simply, and to have their article checked by colleagues before submission. In-house copyediting will be minimal. Non-native speakers of English may choose to make use of a copyediting service.

Help and advice on scientific writing

The abstract is one of the most important parts of a manuscript. For guidance, please visit our page on Writing titles and abstracts for scientific articles.

Tim Albert has produced for BioMed Central a list of tips for writing a scientific manuscript. American Scientist also provides a list of resources for science writing. For more detailed guidance on preparing a manuscript and writing in English, please visit the BioMed Central author academy.

Abbreviations

Abbreviations should be used as sparingly as possible. They should be defined when first used and a list of abbreviations can be provided following the main manuscript text.

Typography

- Please use double line spacing.
- Type the text unjustified, without hyphenating words at line breaks.
- Use hard returns only to end headings and paragraphs, not to rearrange lines.
- Capitalize only the first word, and proper nouns, in the title.
- All pages should be numbered.
- Use the Respiratory Research reference format.
- Footnotes are not allowed, but endnotes are permitted.
- Please do not format the text in multiple columns.
• Greek and other special characters may be included. If you are unable to reproduce a particular special character, please type out the name of the symbol in full. Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF.

Units

SI units should be used throughout (liter and molar are permitted, however).
Appendix D7 : Informed Consent Form

DRAKENSTEIN CHILD LUNG HEALTH STUDY
CONSENT AND INFORMATION SHEET FOR MOTHERS – MAIN COHORT
April 2014

CONSENT FORM AT ENROLMENT
You and your child are invited to take part in a study that is being done in the Drakenstein sub-district, in collaboration with the Universities of Cape Town and Stellenbosch. The following information describes the study and you and your child’s role. Please read this carefully and feel free to ask any questions.

Why is this study being done?
Lung infections and chest problems are common in young children. This study is being done to find out the effect of chest infections in the first year of life on the development of lung disease in children. The study will also look at a number of other factors that may affect your child’s health.

You and your child will be enrolled in the study at the time of his/ her birth. After that, you and your child will be carefully followed up for a few years. You and your baby will be followed regularly at your primary health care clinic and at Paarl Hospital. During these visits, we will assess the health of you and your child by using questionnaires and doing tests. Should your baby get sick with a chest infection, then he/ she will be carefully investigated to try and find out the cause of this infection. This study will help us to better understand why children get chest illness and may help to improve child health.

What must I do if I agree to take part in the study?
If you agree to participate in this study, we will follow you and your child regularly to assess his/ her health. In the first year, we will see you and your child at Paarl hospital at delivery/birth of your child, 6-10 weeks, 1 year and 2 years. We will also follow you and your child at your regular primary health care clinic visits for routine immunizations at 6, 10, 14 weeks, 6 and 9 months. Your child may also be seen every 2 weeks to closely monitor his / her health and to investigate which germs are present in your child’s nose. We will ask you some questions about your child’s health, nutrition, growth and development, and any chest illnesses. We will do regular tests to watch these. These tests are described in more detail below.

We will also ask you and your child to visit Paarl Hospital once a year for a study visit. At this visit, you will be asked some questions about you and your child’s health. Your child will be examined. Tests will be done on you and your child to assess whether there is any chest problem. The tests that may be done on your child are:
Blood tests - these will be to test for allergies or blood problems.
A test of the mucus from the nose (nasopharyngeal swab) to test for infection.
Saliva will be collected to check for germs which may cause pneumonia
A skin test for tuberculosis infection.
A urine test for smoke exposure.
A stool test to check what germs are in the stool.
A set of developmental measures in a subset of infants.
At 7-10 weeks of age a breathing test will be done while your baby is sleeping, to measure the air moving in and out of his/her lungs.
Your child may be asked to undergo a safe, painless brain scan at 2-4 weeks of age
A skin test if your child has a rash

The tests YOU will be asked to complete are:
A blood test to test to check for any other factors that may predispose your child to certain lung illnesses and storage.
A blowing test to check how healthy your lungs are
If you are breast feeding, a small sample of breast milk to test the nutrition in the milk your child is receiving.
A sample of mucus from your nose to check for germs in your nose
Questionnaires about your socioeconomic status and your levels of emotional distress, stress, life events, and drug and alcohol use. If a mental health condition or abuse is suspected, you will be referred to the appropriate local services. You may also be invited to return to undergo more thorough follow-up. This voluntary follow-up session will involve a clinical/psychiatric interview; and a neuropsychological assessment that tests your memory, problem-solving skills, and your attention. You will also be given a questionnaire to complete about your experiences while in the Drakenstein study.

A 5-minute videotaped session of you and your child playing together. This will be used to study how different mothers and their babies play with each other

A urine test for drug and alcohol use

A swab from your vagina which will be taken when you give birth

A stool specimen while you are at the hospital for your child’s birth.

Swabs from your skin and from your cheek at the time when you give birth.

We will only share your test results with primary health care staff if it indicates that you or your child require treatment or further follow up. For some assessments, study staff may follow up with you and provide you with information on where you can seek help, if necessary.

Should your child get sick with a chest infection, then additional tests will be done to try and find out the cause of your child’s illness. The tests that will be done will depend on how sick your child is and what the illness is. These tests may include:

Blood tests to test for infections, at the time of the illness, and again 4-6 weeks afterwards

A test of the mucus from the nose (nasopharyngeal swab) to test for infection

A skin test for tuberculosis infection.

A test of the mucus from the lungs (induced sputum test) for chest infections

A urine test for smoke exposure

Chest X-ray.

Breathing test

A ultrasound test of the lungs

Blood that is drawn from the umbilical cord during birth and at the study visits will be stored for possible further studies including genetic studies (to investigate whether there are particular genes that may predispose a child to pneumonia or other illnesses).

A member of the study team will pay you a visit at your home before you give birth and in the 6 months after your baby is born to collect more information about you and your baby’s living conditions.

What are the benefits of my child being in the study?

You and your child will be closely followed for the first few years of your child’s life. Any medical illness or problem should be found soon after it develops. Your child’s growth and development will be carefully followed. If an illness or problem is found then your child will be promptly investigated and treated. If your child gets sick you will be able to take him/her to your usual health facility, where additional tests to find out the cause of your child’s illness may be done, depending on how sick your child is. If your child requires hospitalisation, then he/she will be hospitalised at Paarl hospital as is usually done. If your child is hospitalised, then one of the study staff will see your child in hospital and additional investigations may be done to try and find out the cause of the illness. Therefore the study offers an opportunity for your child to receive appropriate medical care. The study will also help us to better understand the causes of illness in children, and identify the things that may harm their health. We hope that this will lead to improvements in child health.

What are the risks to my child?

There are no major risks to your child. There may be some discomfort associated with some of the tests we will do. These tests are listed below:

(1) Blood tests
Your child may feel sore when blood samples are taken with a needle. Where possible an anaesthetic cream will be used to dull the pain from the needle. Some bruising may occur, but this is not harmful and will disappear. Only a small amount of blood (not more than 3 teaspoons) will be taken from your child at any time.

(2) Nasopharyngeal swab
A sample of mucus will be taken from your child’s nose, to test for germs that can cause chest infections and to monitor which germs are usually in your child’s nose. Your child may experience minor discomfort when the nasal swab is done. Occasionally it can cause bleeding from the nose, but this is not serious, and usually stops by itself.

(3) TB skin test
A small injection is made on your child’s arm. This is to test whether your child has TB or not, and will be done at regular visits. Your child will experience minor discomfort due to the needle, with the skin test. There may also be irritation of the skin if the test is positive (reactive). This test will need to be checked 2-3 days after the injection is given.

(4) Induced sputum
Your child will be given salt-water through a nebulizer to loosen the mucous in the lungs. Then a sample of that mucus will be suctioned, or your child will be asked to cough up the mucus. Your child may experience a little discomfort while the sputum test is done. He/she may develop some coughing or have a small amount of bleeding from the nose after this. These are not serious. Occasionally this test can cause the airways of the lungs to close. If this occurs your child will be given medicine through an inhaler/nebulizer to open the airways.

(5) Breathing test
This test is done after a child recovers from pneumonia, and at the 6-10 week, 1 year and 2 years follow up visits at Paarl Hospital, while your child is asleep and should not cause any discomfort. While your child is asleep a mask will be put on his/her face and the air going in and out of his/her lungs while breathing will be recorded.

(6) Stool test
This test may be done monthly on your child and then every 6 months after 1 year. Study staff will collect stool from your child’s nappy if passed during a study visit. If there is no stool available, a small tube will be inserted into your child’s bottom and some stool will be sucked out with a syringe. The tube is thin and bendable and is only put in 1-2 centimeters to reach stool. There is a very small chance of bleeding at the rectum right where the tube goes.

(7) Ultrasound test of the lungs
This test will be done if your child develops pneumonia so as to better see how the infection is affecting your child’s lungs. This is a very safe procedure and there are no side effects.

What are the risks to you?
There are no major risks to you. You may feel some discomfort with some of the following tests:

Blood tests
You will experience discomfort when blood samples are taken with a needle. Some bruising may occur, but this is not harmful and will disappear.

(2) Lung function test
This test will be done at each visit to Paarl Hospital. You will be asked to blow into a machine that tests how healthy your lungs are. You will then be given an inhaler with medicine that opens up the chest, and asked to blow into the machine again. You should not experience any discomfort during this procedure. You may feel shaky after the medicine, and your heartbeat may be faster, but this will only last for a short while.

Will I be paid to participate in the study?
No, you will not be paid to participate in this study. If you agree to take part, we will reimburse your transport costs for visits that are not part of your routine antenatal or well baby clinic visits.

Will there be any cost to participate in the study?
No, there will be no cost to you.

How long will my child be in the study?
This consent form is for permission for you and your child to participate in the study for the first year. However, your child will be involved in the study for at least 2 years, with the regular routine clinic visits, as well as hospital visits at 6-10 weeks, 1 year and 2 years. Each year we will ask you again to sign permission for you and your child to continue in the study for another year. You may withdraw from the study at any time. All personal information that you provide, will remain confidential.
Will my child’s participation in the study be confidential?
All information that you provide will be considered confidential, and no mention of you or your child’s name will appear on the stored samples or in any publication in connection with this study. No persons other than the health care workers overseeing your child’s care and the study nurses and doctors will have access to any information that identifies your child personally. All your test results will not be disclosed to anyone other than for the purpose of treating you if there is a problem.

Does my child have to be in the study?
You can choose not to take part in the study. This will not affect the quality of care your child receives. We will ask you to sign a new consent form each year when you visit Paarl hospital. Each year we will explain what we propose to investigate for that year. You will be able to decline to participate at any time should any part of the study be unacceptable to you, you may still take part in the rest of the study.

What do I do if I have any questions?
If you have any questions about this study, you can ask study staff, the Principal Investigator or the lung study doctor at: 021 860 2802. For questions about your rights as a study participant call the Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town, Tel: 021-4066492

Informed Consent
I, ____________________________ understand the information contained in this consent form, as explained to me in a language that I understand and I am prepared to participate in this study, with my child.

I agree to allow study staff to access my medical and hospital records as well as those of my child during the course of the study.

Storage of samples
If any of the samples I have provided for this research project are unused or leftover when the project is completed:

☐ I consent to my samples being stored for future research of any type which has been approved by a Human Research Ethics Committee including for genetic testing

or

☐ I do not consent to my samples being stored for future research

Storage of your child’s samples
If any of the samples my child has provided for this research project are unused or leftover when the project is completed:

☐ I consent to my child’s samples being stored for future research of any type which has been approved by a Human Research Ethics Committee including for genetic testing

or

☐ I do not consent to my child’s samples being stored for future research

2. To be completed by mother:

Mother’s Name: __________________________________________________________

Mother’s Signature: ______________________________________________________

Date: ___________________________________________________________________

3. Study staff providing information: Study staff confirming consent:

Name: __________________________ Name: __________________________

Role in Study: __________________________ Role in Study: __________________________

Signature: __________________________ Signature: __________________________

Date: ___________________________________________________________________

4. If the mother is unable to read or write the entire counselling process must be observed by an independent witness who can then confirm the procedure once the mother has given consent.
Fingerprint of mother:

Witness:
I confirm that I am independent of the study and that I witnessed the entire enrolment counselling process in the home language of the mother.
Name: _____________________________________________________________
Signature: ____________________________________________________________
Date: ________________________________________________________________
# CRF: Maternal Lung Function Testing

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**Bronchodilator**

| 22 Time given (24 hour clock) |         |
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**Spirometry**

<table>
<thead>
<tr>
<th>Test</th>
<th>Time 24.1-26.1</th>
<th>FVC 24.2-27.2</th>
<th>FEV1 24.3-27.3</th>
<th>FEV1/FVC 24.4-27.4</th>
<th>MMEF 24.5-27.5</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Test 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Test 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Test 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td><strong>Best</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Total number of attempts: _______________________________ |         |

**Quality**

<table>
<thead>
<tr>
<th>Effort</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Maximal 9 Maximal Submaximal</td>
<td>0</td>
</tr>
<tr>
<td>Acceptable*</td>
<td></td>
</tr>
<tr>
<td>*meets SOP criteria: start and end of test, free from artefact</td>
<td></td>
</tr>
<tr>
<td>3 Yes 1 No</td>
<td>2</td>
</tr>
<tr>
<td>Repeatable</td>
<td></td>
</tr>
<tr>
<td>&lt;150ml difference best two FVC and FEV1</td>
<td></td>
</tr>
<tr>
<td>3 Yes 3 No</td>
<td>4</td>
</tr>
</tbody>
</table>
**CRF: Maternal Respiratory & Medical Enrolment Form**

**PREGNANCY & CURRENT CONDITION**

1. When was your last menstrual period? [DD / MMM / YYYY]
2. When is your expected date of delivery? [DD / MMM / YYYY]
3. Are you currently well? 
   - Yes
   - No

4. Has a doctor or nurse told you that you have any of the following health problem/s **during this pregnancy**? *Tick all that apply.*
   - Asthma
   - TB
   - Emphysema
   - Chronic Bronchitis
   - Pneumonia
   - Cold/Flu
   - Excessive vomiting
   - Diabetes
   - High blood pressure/eclampsia/pre-eclampsia
   - Pelvic inflammatory disease
   - Heart problem
   - Depression
   - HIV
   - Urine Infection
   - Other
   - (specify): ____________________

5. Are you currently taking medication or was medication prescribed today? 
   - Yes
   - No
   *If yes, record in medication chart.*

6. At the time you became pregnant, were you using any family planning? 
   - Yes
   - No

7. At the time you became pregnant, were you trying to have a baby? 
   - Yes
   - No

---

Please fill in the following information for mother’s previous pregnancies:

*Data abstraction from ANC card.* For “sex” record F or M. For “status” A=alive, NND = Neonatal death; ID = Infant death; IUD = Intra-uterine death.

<table>
<thead>
<tr>
<th>(A) Year</th>
<th>(B) Gestation</th>
<th>(C) Delivery</th>
<th>(D) Weight</th>
<th>(E) Sex</th>
<th>(F) Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>FT / Prem</td>
<td>NVD / CS</td>
<td>M / F</td>
<td>A / NND / ID / IUD</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>FT / Prem</td>
<td>NVD / CS</td>
<td>M / F</td>
<td>A / NND / ID / IUD</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>FT / Prem</td>
<td>NVD / CS</td>
<td>M / F</td>
<td>A / NND / ID / IUD</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>FT / Prem</td>
<td>NVD / CS</td>
<td>M / F</td>
<td>A / NND / ID / IUD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FT / Prem</td>
<td>NVD / CS</td>
<td>M / F</td>
<td>A / NND / ID / IUD</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-----------</td>
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<td>------</td>
<td>-------------------</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviated version.*
# CRF: Socioeconomic Status

Mother Participant ID: __ / __ / __ Date: __ / __ / ___

<table>
<thead>
<tr>
<th>Socioeconomic Status</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 How many children (under 18 years) do you have?</td>
<td>Number: ___________________</td>
</tr>
<tr>
<td>2 How many people normally live in your household? (Include people who live there for more than 6 months of the year)</td>
<td>Number: ___________________</td>
</tr>
</tbody>
</table>
| 3 How many of these are adults over 18 years?                                        | ☐ No Adults  ☐ 1-3 Adults  ☐ More than 3 adults  
(Please specify: ___________) |
| 4 How many of these adults over 18 years are women and how many are men?            |   |
| *If none over 18, please indicate “0”*                                              | 4.1 Number of men: ____________ |
| 5 How many of these are children aged 5 to 18 years?                                 | Number: ___________________ |
| *If none, please write “0”*                                                         | 5.1 Number of girl(s): |
| 6 How many of these children aged 5 to 18 years are girls and how many are boys?    | 6.2 Number of boy(s): |
| *If none between 5 and 18, please indicate “0”*                                     |   |
| 7 How many of these are children younger than 5 years?                               | Number: ____________ |
| *If none, please write “0”*                                                         | 7.1 Number of girl(s): |
| 8 How many of these children younger than 5 years are girls and how many are boys? | 8.2 Number of boy(s): |
| *If none younger than 5, please indicate “0”*                                       |   |
| 9 What is your relationship to each adult or child living with you at home?          | ☐ Your spouse/partner  ☐ Your son or daughter  ☐ Your son-in-law or daughter-in-law  
☐ Your grandchild  ☐ Your parent  ☐ Your parent-in-law  
☐ Your brother or sister  ☐ Your nephew or niece  ☐ Your adopted/foster/step-child |

_99_
| **10** | **What is your Race?** | ☐ Black | ☐ White | ☐ Coloured | ☐ Indian/asian | ☐ Other | ☐ Not related |
| | | ☐ Other | (specify): ______________________ |
| **11** | **What language do you speak at home?** | ☐ English | ☐ Afrikaans | ☐ isiXhosa | ☐ Other | ☐ Not related |
| | | ☐ Other | (specify): ______________________ |
| **12** | **What is your religion?** | ☐ Muslim | ☐ Christian | ☐ Jewish | ☐ None | ☐ Other | ☐ Not related |
| | | ☐ Other | (specify): ______________________ |
| **13** | **Where were you born?** | ☐ In Paarl | ☐ Outside of Paarl, in the Western Cape | ☐ Outside of the Western Cape, in South Africa (eg. Eastern Cape, KwaZulu Natal etc.) | ☐ Please specify: ______________________ |
| | | ☐ Outside of South Africa | ☐ Please specify: ______________________ |
| **14** | **How far did you get in school?** | ☐ No education | ☐ Completed Grade 1 (Sub A) to Grade 5 (Standard 3) | ☐ Completed Grade 6 (Standard 4) to Grade 7 (Standard 5) | ☐ Completed Grade 8 (Standard 6) to Grade 11 (Standard 9), ie. High school without matriculating | ☐ Completed Grade 12 (Standard 10) ie. High school with matriculating | ☐ Part of university/college/post-matric education | ☐ Completed university/college/post-matric education |
| | | ☐ Part of university/college/post-matric education |
| **15** | **What is your current employment situation?** | ☐ Working Now | ☐ Self-employed | ☐ Looking for work: Unemployed | ☐ Temporarily Laid Off | ☐ Other | ☐ Not related |
Abbreviated version.

<table>
<thead>
<tr>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homemaker</td>
</tr>
<tr>
<td>Student</td>
</tr>
<tr>
<td>Illness/sickness</td>
</tr>
<tr>
<td>Disabled</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>(please specify):</td>
</tr>
</tbody>
</table>