HOUSEHOLD AIR POLLUTION AND ADULT PNEUMONIA IN MALAWI

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy by

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Declaration

I declare this thesis to be the result of my own work, except where explicitly stated below. The contributions of others are listed here and described in more detail at the beginning of relevant chapters. The work was conducted at the Liverpool School of Tropical Medicine (LSTM), UK and the Malawi-Liverpool-Wellcome Trust Clinical Research Programme (MLW), UK. My supervisors Dr Kevin Mortimer (LSTM, UK), Dr Ingrid Peterson (MLW, Malawi) and Professor Stephen Gordon (MLW, Malawi) advised on design, conduct, analysis and reporting for all research presented here. The work within this thesis reflects work completed during my Wellcome Trust Clinical PhD Fellowship.

Individuals	**Contribution**	**Chapter**
Dr Deborah Havens (LSTM, UK) and Dr Daniel Pope (University of Liverpool, UK)	Systematic review: protocol design input, title/abstract/paper searching	3
Professor Nigel Bruce (University of Liverpool, UK)	Systematic review: protocol design input	3
Hope Simpson (London School of Hygiene and Tropical Medicine, UK) and Dr Geoffrey Manda (University of Malawi)	Systematic review: title/abstract/paper searching	3
Dr Jamie Rylance (LSTM, UK)	Airway macrophage particulate load study: study conception and design	4
Dr Latifa Patel (formerly Aintree University Hospitals, UK)	Airway macrophage particulate load study: assistance with participant recruitment	4
Dr Andy Cross (University of Liverpool	Airway macrophage particulate load study: assistance with sputum processing and analysis	4
Vincent Doyle (Concern Universal, Malawi), Conor Fox (Clioma Ltd, Malawi), Elizabeth Banda (Clioma Ltd, Malawi), Christa Roth (Fuel and Food Consultants, Germany) and Dr Sean Semple (University of Aberdeen, UK).	Cookstove randomised controlled trial pilot: input into study design	5

James Kachidihu, Howard Bandah and Macfary Kapanga (all Concern Universal, Malawi)	Cookstove randomised controlled trial pilot: data collection	5
Professor Robert Heyderman (formerly MLW, Malawi)	Acute Infection of the Respiratory tract (AIR) Study: input into study design	6
Professor Brian Faragher (LSTM, UK)	AIR study: statistical advice	6
Dr Stephen Aston (Principal Investigator of the MARISO study, MLW, Malawi) and Dr Antonia Ho (Principal Investigator of the BASH-FLU Study)	AIR study: input into study set up and execution, team supervision, data collection and management	6
AIR, BASH-FLU, MARISO and Influenza Surveillance Study teams (all MLW, Malawi)	AIR study: data collection and management	6
Lindsay Zurba (Spirometry Training Services Africa CC, South Africa)	Air study: spirometry interpretation and quality assurance	6
Brigitte Denis, George Selemani, Aaron Mdolo and MLW laboratory staff (all MLW, Malawi)	AIR study: clinical sample analysis	6
Rachel Lloyd (LSTM, UK)	AIR study: data management assistance	6
Dr Nico Nagelkerke (formerly MLW, Malawi)	AIR study: interim analysis	6
Dr Ingrid Peterson (MLW, Malawi)	AIR study: construct of principal components analysis	6
Dr Emanuele Giorgi (Lancaster University, UK)	AIR study: geospatial analysis, data imputation using spatial interpolation and support of multivariate logistic regression analysis	6

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Publications related to work presented in this thesis

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Jary H, Rylance J, Patel L, Gordon SB, Mortimer K. Comparison of methods for the analysis of airway macrophage particulate load from induced sputum, a potential biomarker of air pollution exposure. BMC Pulmonary Medicine. 2015;15:137.

Jary H, Kachidiku J, Banda H, Kapanga M, Doyle JV, Banda E, et al. Feasibility of conducting a randomised controlled trial of a cookstove intervention in rural Malawi. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2014;18(2):240-7.

Jary H, Mallewa J, Nyirenda M, Faragher B, Heyderman R, Peterson I, et al. Study protocol: the effects of air pollution exposure and chronic respiratory disease on pneumonia risk in urban Malawian adults--the Acute Infection of the Respiratory Tract Study (The AIR Study). BMC Pulmonary Medicine. 2015;15:96.

Jary H, Aston S, Ho A, Giorgi E, Kalata N, Nyirenda M, et al. Household air pollution, chronic respiratory disease and pneumonia in Malawian adults: A case-control study [version 1; referees: 2 approved]. Wellcome Open Research. 2017;2:103.

Abstract

Household air pollution and adult pneumonia in Malawi
Hannah Jary

Background: Three billion people worldwide use solid fuels for cooking and heating their homes. The World Health Organization estimates that the resultant household air pollution causes 6.5 million deaths per year, predominantly in low- and middle-income countries. Despite causing half a million deaths per year from childhood pneumonia, the association between household air pollution and pneumonia in adults - a common cause of morbidity and mortality in sub-Saharan Africa – is not established. Studies of household air pollution are limited by difficulties in quantifying exposure levels, contributing to the relative scarcity of evidence. Addressing these methodological challenges would facilitate efforts to obtain the evidence required to reduce this health burden for the world's poorest populations. Focussing particularly in the sub-Saharan country of Malawi, this thesis aims to explore the challenges regarding exposure measurements in resource poor settings, and to provide evidence regarding the role of household air pollution in pneumonia in adults.

Methods: The literature was systematically reviewed to establish the current evidence base for an association between household air pollution and pneumonia in adults. Two prospective studies examining the suitability of potential biomarkers of household air pollution exposure were undertaken: firstly, to establish the feasibility of using airway macrophage particulate load obtained from induced sputum samples as a biomarker; and secondly, to explore whether exhaled carbon monoxide is a suitable biomarker for use in field studies. Finally, a case-control study of HIV-positive and HIV-negative Malawian adults was undertaken to establish the role of household air pollution, and other potential risk factors, in pneumonia.

Results: Eight studies regarding household air pollution and acute lower respiratory tract infection were identified, reporting conflicting data and with limited study quality. The two methods used to calculate airway macrophage particulate load were lengthy, complex and unreliable. Exhaled carbon monoxide tests were easy to use and acceptable to participants in Malawi, but were subsequently found to not correlate with measured air pollution exposures. 145 (117 HIV-positive; 28 HIV-negative) cases and 253 (169 HIV-positive; 84 HIV-negative) controls completed follow up in the case-control study. Household air pollution was not associated with pneumonia in HIV-positive (*e.g.* ambulatory particulate matter adjusted odds ratio [aOR] 1.00 [95% CI 1.00–1.01, p=0.141]) or HIV-negative (*e.g.* aOR 1.00 [95% CI 0.99–1.01, p=0.872]) participants. Chronic respiratory disease was associated with pneumonia in HIV-positive (aOR 28.07 [95% CI 9.29–84.83, p<0.001]) and HIV-negative (aOR 104.27 [95% CI 12.86–852.35, p<0.001]) participants.

Conclusions: There is insufficient evidence in the existing literature to confirm an association between household air pollution and pneumonia in adults. Previous studies have been limited by methodological issues. To address these challenges, this thesis has added to the growing body of literature regarding biomarkers of household air pollution exposure. However, our finding suggest that neither airway macrophage particulate load nor exhaled carbon monoxide are well suited for use at scale in resource poor settings. This thesis reports the largest study of household air pollution and adult pneumonia to date, with detailed exposure and outcome assessments. Although the case-control study sample size was not met, we found no evidence for an association between household air pollution and pneumonia in Malawian adults; further studies should be conducted to ensure that future public health resources are appropriately targeted. Broader solutions, including tackling poverty, malnutrition and chronic respiratory disease, will likely be required to reduce the burden of pneumonia in resource poor settings.

List of abbreviations

8-oxodG	8-oxo-7,8-dihydro-2'-deoxyguanosine
AIDS	Acquired Immunodeficiency Syndrome
AIR	Acute Infection of the Respiratory Tract Study
ALRI	Acute Lower Respiratory Tract Infection
AM	Airway Macrophages
AMPL	Airway Macrophage Particulate Load
aOR	Adjusted Odds Ratio
ATS	American Thoracic Society
BAL	Bronchoalveolar Lavage
BASH-FLU	Burden and Severity of HIV-associated Influenza Study
BMI	Body Mass Index
BOLD	Burden of Obstructive Lung Disease Study
bpm	beats/breaths per minute
CI	Confidence Interval
CO	Carbon Monoxide
COPD	Chronic Obstructive Pulmonary Disease
CRD	Chronic Respiratory Disease
CURB	Pneumonia severity score based on confusion, urea, respiratory rate and blood pressure

CURB65	Pneumonia severity score based on confusion, urea, respiratory rate, blood pressure and age over 65 years
DALYs	Disability Adjusted Life Years
DEP	Diesel Exhaust Particles
DNA	Deoxyribonucleic Acid
eCO	Exhaled carbon monoxide
ELISA	Enzyme-linked Immunosorbent Assay
EMAP II	Endothelial Monocyte-activating Polypeptide-II
FEV_1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IDSA	Infectious Diseases Society of America
Image SXM	Digital image analysis software
ImageJ	Digital image analysis software
IQR	Interquartile Range
ISAAC	International Study of Asthma and Allergies in Children
LSTM	Liverpool School of Tropical Medicine
MARISO	Malawian Adult Lower Respiratory Tract Infection Severity, Aetiology and Outcome Study
MDA	Malondialdehyde

MLW	Malawi-Liverpool-Wellcome Trust Clinical Research Programme
MOST	Pneumonia severity score based on based on male sex, oxygenation, inability to stand and tachycardia.
N/A	Not applicable
NHANES III	The Third National Health and Nutrition Examination Survey
OR	Odds Ratio
PCR	Polymerase chain reaction
PGP	Proline-glycine-proline peptide
PM	Particulate Matter
$PM_{<0.1}$	Particulate Matter less than 0.1µm diameter
PM_{10}	Particulate Matter less than 10µm diameter
$PM_{2.5}$	Particulate Matter less than 2.5µm diameter
ppm	parts per million
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QECH	Queen Elizabeth Central Hospital
RAGE	Receptor for Advanced Glycation End products
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RESPIRE	Randomized Exposure Study of Pollution Indoors and Respiratory Effects Trial
rpm	revolutions per minute

SMRT-CO	Pneumonia severity score based on systolic blood pressure, multilobar chest radiography involvement, respiratory rate, tachycardia, confusion, and oxygenation
SOB	Shortness of Breath
SpO2	Peripheral capillary oxygen saturation
SPSS	Statistical software
STD	Standard Deviation
TB	Tuberculosis
UCB-PATS	University of California, Berkeley - Particle and Temperature Sensor
UK	United Kingdom
USA	United States of America
USD	United States Dollar
WHO	World Health Organization

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1. General Introduction

1.1. Chapter Preface

The World Health Organization (WHO) estimates that 11.6% of all global deaths - 6.5 million deaths - in 2012 were associated with indoor or outdoor air pollution (1). The majority (92%) of people worldwide are exposed to levels of air pollution that exceed recommended limits, according to estimates by WHO generated using data from over 3000 locations around the world (1). Almost 90% of these deaths occur in low- and middle-income countries, and are the result of non-communicable diseases, such as chronic obstructive pulmonary disease (COPD), cardiovascular diseases and lung cancer, as well as infectious diseases, such as respiratory infections (1, 2). This thesis explores air pollution exposures and their adverse health effects, with a particular focus on pneumonia in the sub-Saharan African country of Malawi, where indoor air pollution from household solid fuel burning is high (3). In low-income countries like Malawi, pneumonia has high incidence and mortality rates (4-6). Identification of preventable risk factors for pneumonia, such as air pollution exposure, may help to alleviate this public health burden in the future.

1.2. Household air pollution

Three billion people worldwide are exposed to household air pollution each day due to a reliance on burning solid fuels for domestic energy purposes (2). Essential everyday activities, such as cooking, lead to toxic exposures for individuals – especially women in developing countries – who rely on polluting solid fuels due to poverty. It is women - who traditionally have responsibility for cooking - and the children they care for who are at most risk of the harmful effects of household air pollution.

Deaths from household air pollution affect individuals living in poverty, as highlighted by the similar distributions shown for household air pollution deaths and low income in Figure 1-1 and Figure 1-2. According to the Global Burden of Disease study, household air pollution caused 2.9 million deaths in 2015 and is the eighth most important risk factor for disability adjusted life years (DALYs) worldwide, although this may be an underestimate due to lack of available data on the contribution of household air pollution to ambient air pollution (7). Household air pollution is thought to account for 12% of ambient particulate matter (PM) pollution (8). Globally, the number of deaths and DALYs caused by household air pollution are similar to that caused by ambient air pollution, but in sub-Saharan Africa household air pollution is the major risk factor (9). In Malawi, household air pollution

is the fourth most important risk factor for DALYs, following unsafe sex, childhood undernutrition and unsafe water (7).

These Global Burden of Disease study death and DALY estimates are based on models for the effects of household air pollution on stroke, ischaemic heart disease, COPD, lower respiratory tract infections and cataracts (10). Since the Global Burden of Disease study 2013, lower respiratory tract infections have been incorporated as an outcome for adults as well as children, although due to a paucity of data, the estimates for adults have been based on an integrated exposure-response curve using data for ambient air pollution and tobacco smoke (9, 11). An association between household air pollution and pneumonia in adults is biologically plausible given the known risk in children (12), and the known risk of adult pneumonia associated with ambient air pollution and tobacco smoke exposure (13, 14). However, the extrapolation of data by Global Burden of Disease study has limitations, as discussed below, and direct evidence for the effects of household air pollution would improve these estimates.

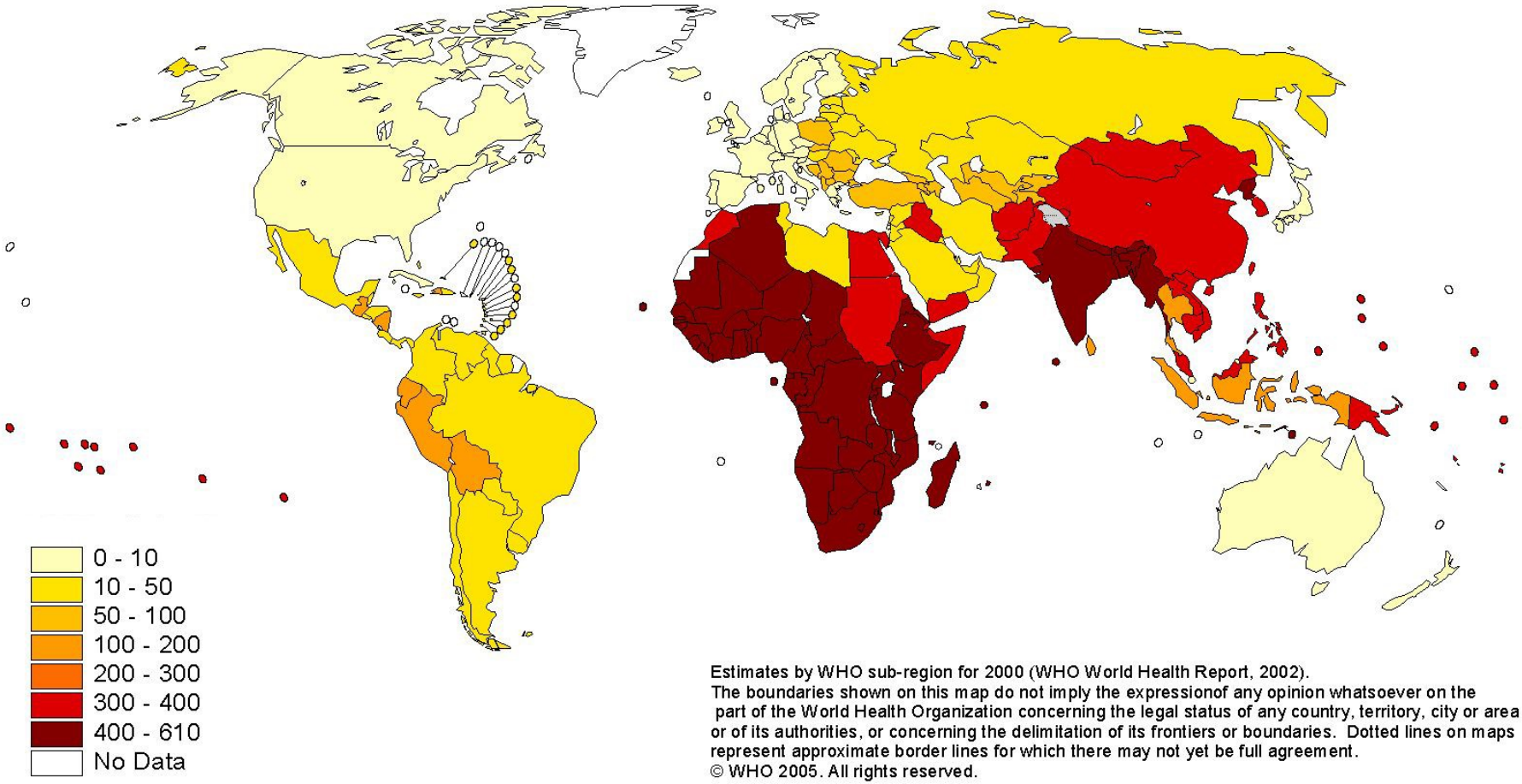

Figure 1-1: WHO map of deaths attributable to household air pollution from solid fuel use.

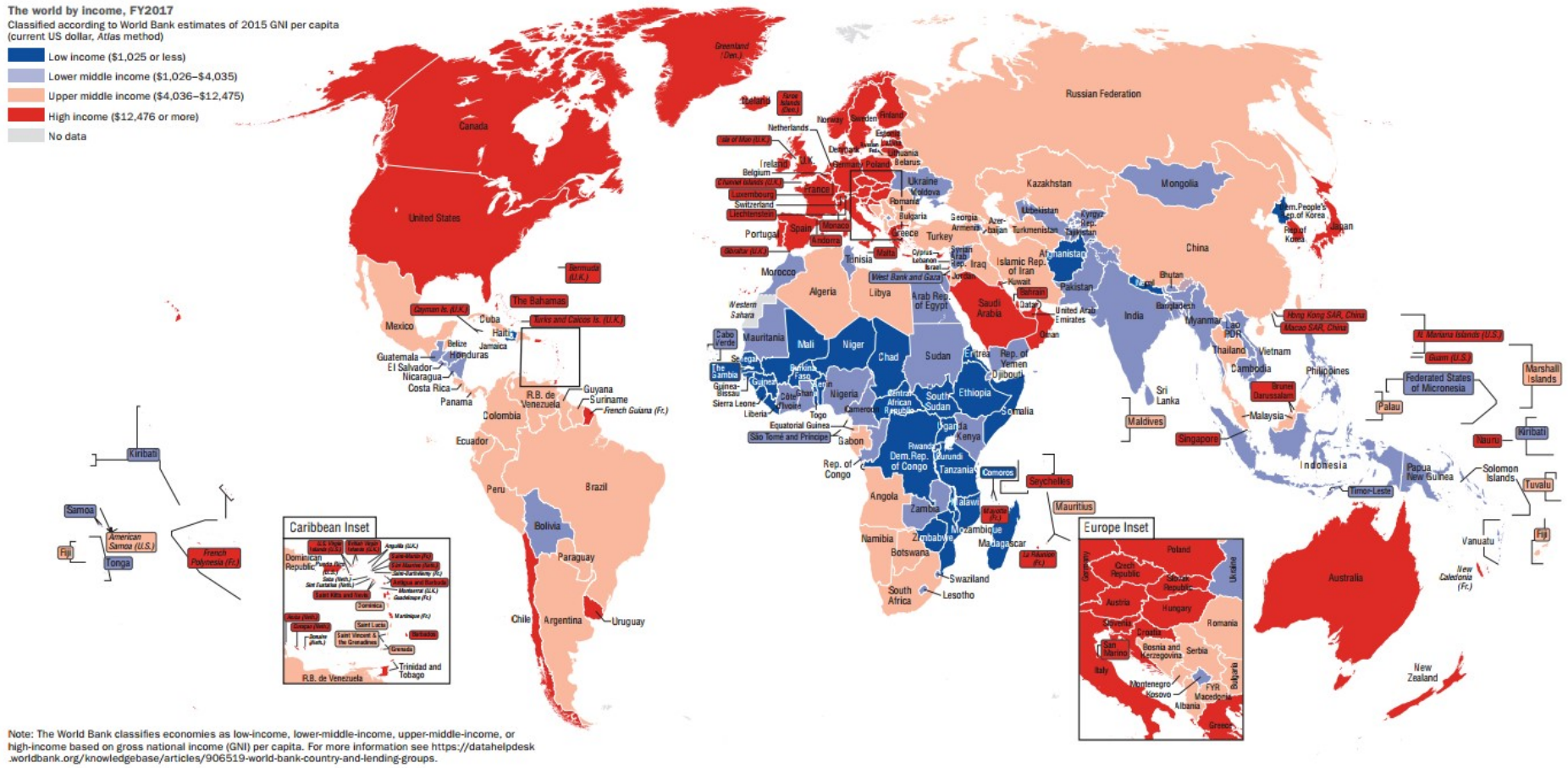

Figure 1-2: World Bank map of global income.

1.3. Pneumonia

With an estimated 291 million incident cases per year (15), acute lower respiratory tract infections (ALRI) cause 2.7 million deaths (16) and the loss of 103 million DALYs per year (17). ALRI are the second leading cause of death worldwide for children under the age of 5, after pre-term birth complications (18). Although DALYs attributed to ALRI have fallen in the past decade, ALRI are the third leading cause of DALYs in all ages (17). Total mortality from ALRI has fallen slightly over the past decade (2.8 million in 2005 to 2.7 million in 2015) (16). Many ALRI deaths are attributable to pneumococcal and *Haemophilus influenzae* type B pneumonia, with 1.5 million and 60 thousand deaths per year in all age groups, respectively (16). Pneumonia is a disease that disproportionately affects people living in poverty, with the highest burden of disease seen in sub-Saharan Africa, South Asia and parts of Latin America.

Data regarding the incidence of adult pneumonia are scarce for sub-Saharan Africa, but estimates of 4 million adult ALRI episodes per year have been made using epidemiological models (19). Pneumonia is a common cause of adult admissions to hospital in Malawi: 17% and 16% of all adult medical admissions to Queen Elizabeth Central Hospital (QECH), Blantyre in 1973 (pre-Human Immunodeficiency Virus (HIV) era) and 2013 (in-patient HIV prevalence 26%) respectively (5, 20). Adult pneumonia mortality in sub-Saharan Africa varies between 6-14.5% (6, 21, 22). Although this figure is comparable to that seen in many European studies, a noticeably younger population is affected in sub-Saharan Africa with over 50% of adult deaths seen in under 35 year olds (6). ALRI are the third leading cause of DALYs in Malawi, following HIV infection and malaria (17).

Pneumonia risk is increased up to 25-fold in individuals with HIV infection in the absence of antiretroviral therapy (23-26), and the risk of invasive pneumococcal disease is increased up to 100-fold (27). Even with established ART, there is 35 times the risk of invasive pneumococcal disease in individuals with HIV compared to those without (28). In settings like Malawi, high HIV prevalence has a major impact on pneumonia incidence, management and treatment (29). Pneumonia in adults who are HIV-seropositive can commonly be complicated by HIV associated coinfections, in particular, tuberculosis.

There are complex relationships between poverty, household air pollution, HIV infection and pneumonia risk. In Malawi - where all of these factors are highly prevalent - if an association between pneumonia and household air pollution is established, then the attributable risk of household air pollution for pneumonia is potentially high. If this is the case, reductions in household air pollution may provide an opportunity to address this preventable health burden in a vulnerable population.

1.4. Study setting

1.4.1. Malawi

Much of the work presented in this thesis was conducted in Malawi, in particular the studies described in Chapters 5 and 6. In this section, I describe some of the characteristics of Malawi and some of the issues faced by the country which have impacted on the research presented in this thesis.

Malawi is a landlocked country situated in southern central Africa, a member of the Southern African Development Community. The country spans 118,484km^2 along the Great Rift Valley, including Africa's third largest lake – Lake Malawi. With an estimated population of 16.3 million people in 2015 (30, 31), Malawi has a population density of 173 persons/km^2(30). This is set to increase further, with an annual live birth rate of 651700 per year and an annual death rate of 147600 per year (32); the population is predicted to reach 37 million by 2050 (33). The majority (84%) of Malawi's population live in rural areas, predominantly in the densely populated Southern Region.

Life expectancy in Malawi has improved dramatically in recent years, mainly a result of improvements in child and maternal health, as well as the introduction of antiretroviral treatments for HIV infection. However, healthy life expectancy at birth is only 50 years and the under-five mortality rate is 68 per 1000 live births (32). Improvements in mortality from infectious causes have been made – although HIV/Acquired Immunodeficiency Syndrome (AIDS) and lower respiratory infections remain the two leading causes of death – but recent years have seen an increase in the number of deaths resulting from non-communicable diseases, including stroke and cardiovascular disease (32). National HIV prevalence is estimated to be 10.8% but this is as high as 17.4% in urban areas (34). Of those with HIV infection eligible to be on antiretroviral therapy in 2012, 69% were receiving treatment (35).

Having fallen sharply in recent years, Malawi's gross domestic product per capita in 2015 was $372, making Malawi the fourth lowest ranking country worldwide according to World Bank estimates (36). Of this gross domestic product, 8.3% is spent on health (35). Malawi's national budget is heavily dependent on foreign aid, which is contingent on political stability: the consequences of this became apparent when aid was withdrawn following the "Cashgate" corruption scandal in 2013[1] (37). With 61.6% of the population living below the poverty line (2007-2011, (34)) and the majority of

[1] "Cashgate" was Malawi's biggest financial scandal, with an estimated $250 million USD lost through allegedly fraudulent payments to businessmen.

individuals dependent on subsistence farming, families have little resilience when faced with additional challenges. This economic instability can have consequences such as national fuel shortages, which impacted on research activities for the pilot study described in Chapter 5. Poor national infrastructure, particularly in rural areas, means that the country is especially vulnerable in the event of natural disasters. Two national emergencies have been declared in Malawi during the course of this PhD: the first following devastating flooding in 2015, which affected an estimated 1.1 million people, displaced 230,000 people and destroyed much of the country's harvest (38); the second following widespread drought in 2016, which resulted in a 96% reduction in maize production in the worst affected districts of the country (39), leaving 6.5 million people food insecure (40). These natural disasters impacted on the case-control study described in Chapter 6, by affecting participant recruitment rates.

Given the lack of national infrastructure for providing energy solutions, and the lack of personal finances, 95% of Malawians depend on cheap solid fuels, such as wood and charcoal, for their domestic energy sources (3, 41). With rapidly increasing population growth, this puts Malawi's natural resources under significant demand with deforestation already widespread across the country: solid fuels are not a sustainable solution for Malawi's energy requirements. Solid fuel use, and the resultant air pollution exposure and health implications, are the focus of this thesis and will be discussed in more detail below.

1.4.1.1. Blantyre

The case-control study presented in Chapter 6 was conducted in Blantyre, Malawi's second city. Whilst Lilongwe in the Central Region is the country's political capital, Blantyre is the financial, commercial and manufacturing centre of Malawi and is located in the Southern Region. Blantyre City Council estimates a population of 884,000 overnight, rising to over 1 million during the day time as a result of a daily influx of workers (30). Between 60-75% of Blantyre's population are estimated to live in informal settlements. The rainy season is from October to April and the dry season is from May to October, although "chiperoni" rains (light drizzle) occur from May to July (30). The Southern Region, including Blantyre, is mainly inhabited by people from the Chewa ethnic group, of the Bantu people.

1.4.1.2. Ntcheu district

130km north of Blantyre, along the border with Mozambique and halfway to Lilongwe, lies the rural district of Ntcheu in the Central Region of Malawi. Ntcheu town, where the pilot study described in Chapter 5 was conducted, is the administrative capital of the district and where Ntcheu District Hospital is located. The district has a population of approximately 475,000 in an area of 3,424km^2

according to the 2008 Malawi population census. The inhabitants of Ntcheu are predominantly Ngoni people. The region is known for vegetable production.

1.4.2. Queen Elizabeth Central Hospital

QECH, Blantyre is a government-funded tertiary-referral hospital, the largest in Malawi with approximately 1200 beds. It is adjoined by a large medical school campus for the College of Medicine, University of Malawi. QECH provides healthcare free at the point-of-care to the population of greater Blantyre (population 1.3 million) and the surrounding districts, receiving referrals from community health centres and district hospitals.

Adult patients are initially assessed by medical or clinical officer staff in the Adult Emergency and Trauma Centre. Patients requiring admission to the medical department will usually be assessed by the on-call medical team (intern or registrar, with possible review by the consultant) before transfer to one of two general medical wards (single-sex) or the tuberculosis ward. The general medical wards each have approximately 60 beds (although patient numbers frequently exceed 100, with mattresses on the floor provided for those without beds) in addition to a 6-bed high dependency unit, where increased nursing-ratios and supplementary oxygen via oxygen concentrators are available.

Nurse staffing levels on the wards are generally low, with all of the patient's personal care being provided by their 'guardian' (usually a friend or relative who has been appointed to care for the patient for the duration of their inpatient stay). General hygiene on the wards is poor, with frequent water shortages, broken lavatories, and lack of soap or disinfectant. Consultant-led ward rounds are conducted twice a week on all medical wards, with interim care provided by junior doctors, clinical officers or medical students. Basic radiological (x-rays, ultrasounds and limited magnetic resonance imaging) and laboratory (basic haematological and biochemistry blood tests, and tuberculosis screening) diagnostics are provided by the hospital or College of Medicine, but it is not uncommon for these to be unavailable due to broken equipment or lack of reagents. Routine microbiological diagnostics (including blood cultures and cerebrospinal fluid culture and analysis) are provided by the Malawi-Liverpool-Wellcome Trust Clinical Research Programme (MLW) laboratories, which are located on the QECH site. Therapeutic options are limited to a relatively small number of essential medicines, which are in theory provided free of charge to the patient, but are frequently unavailable at the hospital pharmacy (including antibiotics, analgesia, cardiovascular medications and insulin). If the patient can afford to, they may be able to pay for diagnostic tests or medicines elsewhere in Blantyre, but for the majority of patients this is not an option.

1.5. Thesis overview

1.5.1. Thesis hypothesis

The aim of this thesis is to explore the respiratory health effects of household air pollution on adults – with a particular focus on pneumonia in Malawi – and some of the methodological issues surrounding research of household air pollution in a sub-Saharan African setting. The overarching hypothesis is that household air pollution is a preventable risk factor for pneumonia, which if confirmed, provides an opportunity to reduce the burden of disease in sub-Saharan Africa using interventions to reduce household air pollution exposure. This thesis also aims to evaluate the use of field biomarkers for household air pollution exposure which will improve capacity to conduct future exposure and intervention studies.

1.5.2. Key research questions

This thesis addresses the following key research questions:

- Is research of household air pollution and its's health effects feasible in Malawi, and what lessons can we learn to improve research implementation?
- Is there a biomarker of household air pollution that is practically useful in a sub-Saharan African setting?
- What is the existing evidence of an association between household air pollution and pneumonia in adults?
- What are the levels of exposure to air pollution in urban Malawi?
- Is household air pollution a risk factor for pneumonia in adults in Malawi, and what are the other potentially avoidable risk factors?

1.5.3. Thesis Outline

The global burden of pneumonia and of air pollution exposures have been outlined in this introductory chapter; this thesis focuses on these two important issues by exploring the relationship between pneumonia in adults and their exposure to household air pollution. Chapter 2 provides a review of the existing evidence for the health effects of air pollution and of current understanding regarding the risk factors for pneumonia. This is followed by a more detailed systematic review focusing on the evidence of an association between household air pollution and ALRI in adults in Chapter 3. Chapters 4 and 5 explore some of the issues regarding conducting research on this topic: firstly, reporting on the findings of a feasibility study regarding the implementation of a household air pollution intervention trial, and secondly, regarding the challenges of developing field-usable biomarkers of exposure with a focus on airway macrophage particulate load. Chapter 6 reports on

the findings of a case-control study in Malawi, examining potentially avoidable risk factors for pneumonia in urban dwelling adults, with a particular focus on the role of air pollution exposure. The thesis concludes with a summary of these findings, and a discussion of the implications for future public health interventions and research.

2. Literature Reviews: Air Pollution and Pneumonia

2.1. Chapter overview

In this chapter, I summarise the current literature for the two major topics of this thesis: air pollution and pneumonia.

Air pollution is the fourth most important risk factor for DALYs worldwide (7), the exposure of interest in this thesis and the focus of the first section of this chapter. Firstly, I provide background information regarding different types of air pollution – including their sources, constituents and factors affecting exposure – and then discuss the health risks associated with air pollution exposure. This is followed by an overview of the factors to be considered when measuring air pollution exposure and air pollution reduction strategies. Ambient, occupational and household air pollution, as well as tobacco smoke exposure, have each been demonstrated to be associated with poor health outcomes, and so are discussed in turn in this section. However, given the heavy reliance of solid fuels in Malawian homes as discussed above, and the relative lack of industrialisation or heavy traffic in Malawi, the major focus of this and subsequent chapters will be household air pollution.

In the second section of this chapter, I summarise literature regarding pneumonia, the disease outcome of interest in this thesis, which results in the death of 2.7 million adults and children per year (16). I provide a background details regarding the pathogenesis, classification and prognosis of pneumonia. I then summarise the current evidence regarding risk factors for community acquired pneumonia, and provide an overview of the limited literature describing pneumonia in adults in sub-Saharan Africa.

2.2. Literature Review: Air Pollution

2.2.1. Ambient air pollution

Ambient air pollution, which refers to outdoor air pollution, has long been associated with significant health problems. The infamous Great Smog of London 1952, which was later estimated to have been responsible for the death of 12,000 people (42), provided the impetus for modern environmental and public health research and legislation with the aim of improving air quality. Despite these measures, the WHO recently estimated that 3 million premature deaths occur annually worldwide as a result of continuing air pollution (43). The impact of ambient air pollution is therefore a major cause of concern worldwide and has been a topic of extensive research over recent decades.

2.2.1.1. Sources of ambient air pollution

Ambient air pollution, which is primarily caused by transport engines, industrial emissions, burning of biomass and natural sources, is typically highest in urban areas.

Particulate matter (PM) can be classified according to their mode of formation: primary PM are emitted directly from their source into the air; secondary PM are formed from gaseous precursors such as sulphur dioxide, nitrogen oxides and ammonia. Both primary and secondary PM can occur from man-made sources (anthropogenic) – such as combustion engines, solid fuel combustion for household or industrial purposes, agriculture, mining and manufacturing of bricks, cement or ceramics – or from natural sources (non-anthropogenic), including dust storms, forest fires and volcanic eruptions. Dust and soil re-suspension contribute to PM in particularly arid areas. Diesel engines produce up to 40 times more PM_{10} (particulate matter <10µm diameter) than petrol engines with catalytic converters of a similar power (44). Although engines have become more efficient, the number of miles travelled by trucks - major contributors to ambient PM - rose from 28,854 million to 170,246 million between 1960 and 2015 in the USA, thus highlighting ongoing difficulties with traffic pollution (45).

Ozone is formed by a photochemical reaction between sunlight and pollutants such as nitrogen oxides or volatile organic compounds, and so occurs at high levels during sunny periods. Nitrogen dioxide and sulphur dioxide are primarily generated by fossil-fuel combustion, for power generation, domestic heating and vehicle engines.

2.2.1.2. Exposure to ambient air pollution

More than 80% of people living in urban areas with air pollution monitoring are exposed to air pollution levels that exceed WHO guidelines; in low- and middle-income countries the situation is worse, with 98% of cities with more than 100,000 inhabitants not meeting WHO air quality guidelines (46). Much of this burden is in Asia, but parts of southern sub-Saharan Africa are also severely affected (46). In Asian cities selected by the WHO, annual average suspended PM concentrations ranged between 35-220µg/m^3, compared to 15-60µg/m^3 in European and North American cities (47).

2.2.1.3. Constituents of ambient air pollution

Ambient air pollution consists of a complex mix of gaseous, liquid suspensions and PM. Four classical indicators of pollution - PM, nitrogen dioxide, sulphur dioxide and ozone - are most commonly studied, and each demonstrate differing trends which are used to characterise air quality (47).

PM, which consists of a mixture of solid and liquid particles, has been shown to affect more people worldwide than any other kind of pollution (43). $PM_{2.5}$ (particulate matter <2.5µm diameter) constitutes 50-70% of PM_{10} across most of Europe (48). The constituents of PM vary greatly by geographical setting, as different sources of PM generate different chemical compositions (49): for example, high black carbon content in PM generated from coal fired power stations, high "crustal matter" in re-suspended desert sand, and diesel exhaust particles (DEP) form a major proportion of PM from vehicular emissions (50).

2.2.2. Occupational air pollution

Individuals exposed in the workplace to airborne contaminants, which may be aerosols (including airborne dusts, mists, fumes and smoke) or gaseous (gases or vapours), are at risk of occupational disease. Globally, a wide variety of occupations lead to such exposures, resulting in acute and chronic diseases or death, as well as a significant economic burden to employers and society. Many countries have implemented strict regulation of workplace exposure to a number of hazardous agents, and prevention of exposure can often be low-cost. Despite this, workplace exposures remain high and unregulated in many parts of the world, leaving vulnerable workers at risk of occupational diseases.

Airborne dust is generated by a variety of occupations, such as: mineral dusts (including silica) from mining quarrying and construction; metallic dusts from manufacturing or metal-industry; chemical dusts in agriculture, manufacturing and pharmaceutical industries; vegetable dusts from agriculture and food processing, and moulds and spores from farming and food industries. Airborne dusts are solid particles with an aerodynamic diameter of less than 100µm, which can be inhaled. Non-soluble dust particles deposited in the airways may cause a local reaction: the depth of inhalation, and therefore site of reaction, will depend on the size of the particle and breathing pattern. Soluble particles, for example lead, may dissolve where it is deposited and be absorbed into the blood stream causing systemic disease. Airborne dusts are known to cause occupational asthma, COPD and pneumoconiosis, a range of restrictive lung diseases characterised by inflammation and fibrosis.

2.2.3. Household air pollution

Household air pollution refers to the presence of airborne pollutants inside the home resulting from the burning of fuel for domestic purposes. Almost half the world's population are exposed to household air pollution, usually a result of poverty leading to reliance on cheap but polluting fuels (2). Between 3 and 4 million deaths per year are thought to be caused by exposure to household air pollution due to the effect on a wide range of diseases (9, 51): the evidence for this, and details regarding specific disease associations, are discussed in section 2.2.5.3. In this section, I describe an

overview of household air pollution including the major sources, factors which effect exposures and the chemical composition of household air pollution.

In addition to the health effects of household air pollution (discussed in section 2.2.5.3.), there are other important consequences of using solid fuel for cooking. Deforestation as a result of using firewood or charcoal for domestic energy is a major problem in many countries, leading to changes in animal and plant habitats (which may impact on food sources), soil erosion (increasing the risk of landslides) and climate change (52). Fuel gathering is time consuming, resulting in less time for education or economic activities (51). As it is often women or children who are responsible for fuel gathering, it is women and children who are deprived of schooling and business opportunities. They are also vulnerable to physical attacks when walking long distances from their home to find fuel (52).

2.2.3.1. Sources of household air pollution

Any form of domestic energy consumption, whether it be for cooking, heating or lighting, can lead to household air pollution if polluting fuels are used, but it is cooking that requires the most fuel consumption worldwide. Household air pollution is usually a result of burning solid fuels, such as biomass fuels (including wood, charcoal, plant matter and animal dung) or coal, but may also be caused by burning liquid fuels including kerosene or paraffin.

Choice of fuel for any given household depends on a variety of factors: cost, tax and subsidies, local availability, energy requirements, household characteristics, cooking behaviours, cultural traditions, and knowledge (53). Fuel use therefore varies widely across different populations in different geographical settings and seasons. For example, coal use is extremely common in China, wood burning is common in much of rural sub-Saharan Africa and Latin America, whereas charcoal is most commonly used in many sub-Saharan African cities. In parts of Asia (including India) and parts of northern Africa, pastoralist communities use dried animal faeces – often cow or buffalo dung – to make ‘dung cakes’, known as chulha or uple, as a cheap and readily available form of bio-fuel.

Cost of fuel plays a key role in determining fuel use, with the trade-off for an affordable price being the quality and “dirtiness” of a fuel: generally the cheaper a fuel is, the more polluting it is. As a consequence, it is the world’s poorest populations who are exposed to the highest levels of air pollution as they rely on dirty fuels with poor combustibility. As prosperity increases, fuel use becomes cleaner, as shown in the Energy Ladder (Figure 2-1) (54); electricity at the top of the ladder causes no household pollution at the point of use, but is only available to the richest of the world’s population.

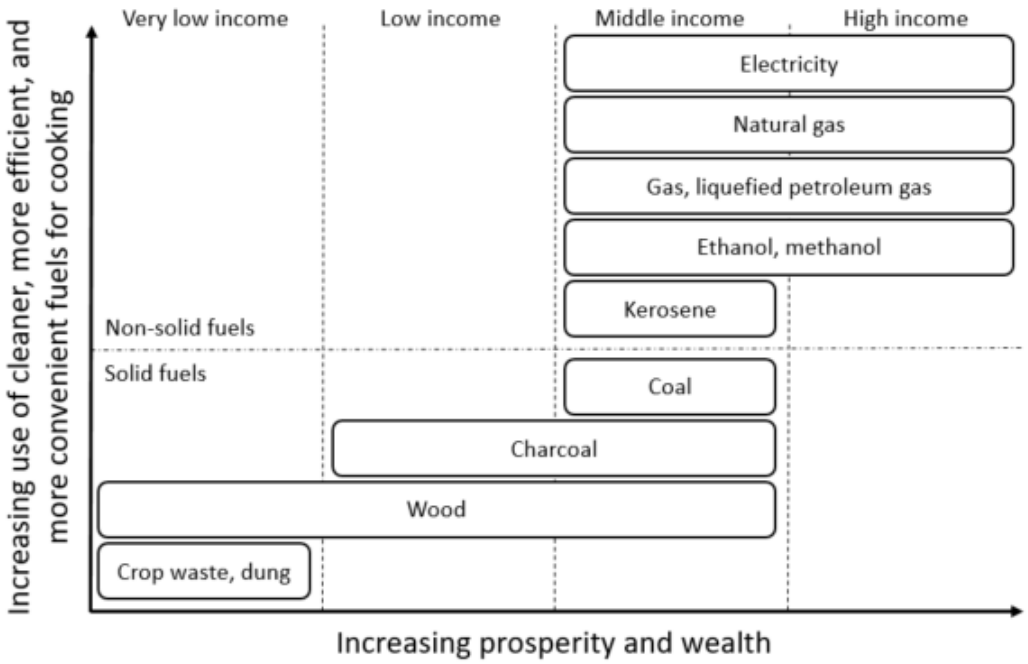

Figure 2-1: The energy ladder: household energy and development are inextricably linked.
Adapted from Rehfuess, WHO, 2006 (55).

Household air pollution may also result from sources other than combustion fuels, such as the burning of mosquito coils or incense sticks. Around 2 billion people burn mosquito coils worldwide, particularly in developing countries, to prevent bites from mosquitoes (56). It is estimated that burning a mosquito coil can release $PM_{2.5}$ equivalent to burning 75-137 cigarettes (57), which can result in a range of respiratory effects (58). Incense is typically burnt for religious or ceremonial reasons in many developing countries, resulting in the release of a variety of pollutants known to cause harm, although the evidence for respiratory health effects is inconsistent (58).

2.2.3.2. Exposure to household air pollution

In addition to choice of fuel, an individual's exposure to household air pollution is dependent on a number of other factors: cooking behaviours, stove type, food choices, ventilation and season (59). Due to traditional domestic roles in many parts of the world, it is women and children who are most exposed to household air pollution (60). In many cultures, it is women who are responsible for both cooking and childcare, so whilst the mother is tending the fire the young child is playing close by or is being carried on the mother's back.

The type of stove or fire used to burn the fuel also affects an individual's exposure. An open or "three-stone" fire (an open fire is built in between 3 large stones, which are used for resting the cooking pot on) is the traditional cooking method in many parts of the world (60). However, this is

an inefficient way of burning fuel, as much of the heat is lost via the open sides of the fire and the airflow cannot be controlled to maximise combustion efficiency. Inefficient combustion leads to higher levels of waste product emissions, and longer cooking times are required due to the amount of energy being lost, prolonging the periods of exposure to pollution (61). Combustion efficiency can be improved by using designed technology, such as a cookstove. Many cookstove designs are available, from simple locally produced clay stoves to fan-assisted stoves, each of which have their own advantages and disadvantages and are described in more detail in section 2.2.7.2. A household's choice of cookstove will depend on the type of food that is typically cooked and cultural cooking practices – such as a preference for roasting, boiling, steaming or stewing - as well as the cost and availability of the stove itself (62, 63).

Ventilation and seasonal variations in weather also play a role in exposure (62). In some parts of the world, exposure may be lower during the dry season as individuals are more likely to cook outdoors than in the rainy season, or exposure may be higher during cold seasons when fuel is burnt indoors for heating. If cooking indoors, an individual's exposure to household air pollution is affected by the design of the house, and whether or not the stove has a chimney or flue. Maintenance of chimneys is also required and if neglected can lead to high levels of household air pollution. House design varies according to socioeconomic status, geography and climate, but often cooking in the simplest mud and thatch homes is performed in small rooms with few windows or doors for ventilation, and no chimney (64). In very poor communities, the cooking may take place in the same room where the family live and sleep, meaning that pollution exposure is high throughout the day and night. In some populations, households prefer to cook indoors as the smoke prevents insects from living inside the thatched roof. Ventilation may also be deliberately reduced to conserve energy, particularly in cold climates.

Other behavioural factors also play a role in exposure, such as cooking practices (for example, using a lid on a cooking pot will reduce cooking time and therefore exposure), time spent near the fire, and drying of fuel before use. Exposure to kerosene pollution has been shown to be higher for men in some communities, where men socialise together in the evenings, around a burning kerosene light, away from the women (65). Men are also typically exposed to household air pollution when they are working away from their home, and therefore take over responsibility for cooking.

2.2.3.3. Constituents of household air pollution

Pollution resulting from the burning of fuels comprises of a complex mixture of solid, liquid vapour and gaseous constituents, including PM, inorganic gases, hydrocarbons, free radicals, chlorinated organics and oxygenated organics (66). The relative proportions of the constituents of pollution vary

according to the fuel type, fuel quality (*e.g.* moist wood vs dry wood) and the combustion conditions. Over 200 distinct organic compounds have been detected in wood smoke (67). Many components of household air pollution – some of which are listed in Table 2-1 – are known to be toxic or irritant to humans, although there is insufficient evidence to know which specific

Table 2-1: Constituents of household air pollution and their mechanisms of actions and health effects (not exhaustive).

Adapted from Bruce *et al,* 2006, Naeher *et al,* 2007 and Fullerton, 2011 (60, 66, 68).

Pollutant	Mechanism	Potential health effects
Particulate matter (PM_{10} and $PM_{2.5}$)	• Acute: bronchial irritation and increased reactivity, inflammation • Reduced mucociliary clearance • Reduced macrophage response and reduced local immunity • Oxidative stress • May be allergenic	• Wheezing, exacerbation of asthma • Chronic bronchitis and COPD • Exacerbation of COPD
Carbon Monoxide	• Binding with haemoglobin to produce carboxyhaemoglobin, resulting in tissue hypoxia and cellular death • Asphyxiant • Neurotoxicity	• Low birth weight • Increased perinatal deaths
Hydrocarbons *e.g.* polycyclic aromatic hydrocarbons (*e.g.* benzo[a]pyrene)	• Carcinogenic • Mutagenic • Irritant	• Lung upper respiratory cancers • Cardiovascular disease
Nitrogen dioxide	• Irritant • Acute exposure increases bronchial reactivity • Longer term exposure increases susceptibility to bacterial and viral lung infections	• Wheezing and exacerbations of asthma • Respiratory infections • Reduced lung function in children • Acute or chronic bronchitis
Sulphur dioxide	• Acute exposure increases bronchial reactivity • Longer term difficult to dissociate from effects of particles	• Wheezing and exacerbations of asthma • Exacerbation of COPD and cardiovascular disease
Condensates including polycyclic aromatics and metal ions	• Absorption of toxins into ocular lens leading to oxidative changes	• Cataract
Oxygenated organics *e.g.* aldehydes, phenols	• Irritant • Carcinogenic • Mutagenic • Teratogenic	• Bronchoconstriction

PM_{10}: particulate matter <10µm diameter; $PM_{2.5}$: particulate matter <2.5µm diameter; COPD: chronic obstructive pulmonary disease.

components are responsible for the disease burden caused by household air pollution, and that may vary according to the disease (60).

Carbon monoxide (CO) and nitrogen dioxide are two of the principal gaseous components of household air pollution, and are known to have harmful effects. PM are thought to be an important indicator for the health effects of pollution (60), and are classified by their size. PM_{10} are PM with a diameter of less than 10µm, $PM_{2.5}$ have a diameter of less than 2.5µm, and $PM_{<0.1}$ are "ultrafine particles" with a diameter below 0.1 µm. The smaller the size of the particle, the greater its potential to cause adverse health effects: it remains airborne for longer; the small size allows deeper inhalation into the lungs, evading the mucociliary defence system; and the large surface area-to-mass ratio means that more toxic compounds can be absorbed onto the surface of the carbonaceous core (43). However, this size classification does not take into consideration the chemical composition of PM, which varies according to the specific fuel type and combustion conditions (66). It is important to note that much of the evidence for the health effects of PM is derived from evidence for fossil fuel combustion: the composition of biomass fuel PM differs to that of fossil fuel PM and therefore the impact on health may also be different. Not only does the relative proportion of PM of different sizes vary depending on the combustion source, but there are also enormous number of possible chemical species associated with the particles which can be used to distinguish between sources (69). For example, particles derived from fossil fuels with a high mass of aluminium, chloride, and chromium are likely to be derived from fossil fuel combustion, whereas high potassium, ammonium and sulphate content is associated with biomass burning (70).

2.2.4. Tobacco smoke

Given the relatively low prevalence of cigarette smoking and relatively high prevalence of household air pollution exposure in Malawi (41), tobacco smoke is not a major focus of this thesis. However, tobacco smoke is an important confounder in studies of the health effects of pollution exposure. Furthermore, the wealth of evidence for the health risks of tobacco smoke has played an important role in informing the literature on household air pollution. For these reasons, some key details regarding tobacco smoke are included here but further detail is beyond the scope of this chapter.

Global Burden of Disease study 2015 estimates that 6.4 million people die per year as a result of tobacco smoke, which is the 2nd and 9th most important risk factor for DALYs worldwide, in men and women respectively (7). Approximately 10% of deaths are in non-smoking individuals who are exposed to second-hand tobacco smoke (71). Active and passive exposure to tobacco smoke is well documented to be associated with a wide range of adverse health effects, including respiratory tract infections (such as pneumonia and tuberculosis), chronic respiratory diseases (such as COPD,

bronchiectasis and exacerbations of asthma), numerous cancers and cardiovascular diseases (7). Tobacco smoke exposure is the single most important risk factor for pneumonia in immunocompetent adults (72), and possible mechanisms include structural changes to the respiratory tract and impaired cell-mediated and humoral immune responses (73). Of the world's 1 billion smokers, 80% live in low- and middle-income countries (71). However, in a recent study in urban Malawi, only 10% of adults reported ever smoking (9.2% of men and 0.7% of women were current smokers), and only 2% of adults reported a pack-year history of 10 years or more (41). According to the Malawi Demographic and Health Survey 2015-16, 0.1% of women (age 15-49 years) and 11.9% of men (age 15-49 years) in urban areas in Malawi smoke cigarettes (74). Smoking increases with age; the highest prevalence of cigarette smoking in women is in 45-49 year olds, and in men it most common in 40-44 year olds. For both genders, smoking is most common amongst those in the lowest socioeconomic quintile and with no education. Amongst the 7.9% of men in urban areas who smoke cigarettes daily, 41% smoke less than 5 cigarettes per day and none smoke more than 24 cigarettes per day. The overall prevalence of smoking in Malawi (aged 15-49 years, rural and urban) has decreased from 23% in 2000 to 13% in 2015-16, which is in contrast to the concerning epidemic of increasing smoking prevalence which is currently seen Africa (74, 75). It is important to keep monitoring these trends in Malawi to ensure that exposure to this known risk factor for mortality does not increase as has been seen elsewhere in Africa.

Tobacco smoke is essentially a form of biomass smoke, and combustion of this plant matter generates PM and other pollutants including irritant gases, similar to those found in household air pollution. It is likely that there will be some differences in health effects between tobacco smoke exposure and other biomass smoke exposures due to variation in the exact chemical composition of the combustion products and the differences in the mode of exposure. Nonetheless, in the absence of specific data regarding other forms of biomass, knowledge of tobacco smoke exposures provide useful insight into the potential health effects of household air pollution (10). It is thought that the health risks of household air pollution lie somewhere between the risks of active and passive tobacco smoking.

2.2.5. Health effects of air pollution exposure

In this section, I describe the current evidence for the health effects of the main sources of air pollution, with a focus on respiratory and cardiovascular disease – the major outcomes of interest for air pollution exposure. I predominantly describe the epidemiological evidence and, for pneumonia (the focus of this thesis), I also provide an overview of studies of mechanisms of disease. The health effects of each type of air pollution exposure are described separately here as, although

similar and overlapping, the health effects are distinct from each other due to the differences in pollution constituents and exposures, as discussed above.

2.2.5.1. Health effects of ambient air pollution

There has been a wealth of evidence documented for the health effects of ambient pollution in recent decades, particularly since the London Smog 1952, which has led to widespread efforts to reduce ambient air pollution exposures. Despite this, 3 million premature deaths were estimated to be caused by ambient air pollution in 2012, according to the WHO, with 88% of deaths occurring in low- and middle-income countries (43). The WHO estimates that ischaemic heart disease and strokes are responsible for 80% of ambient air pollution deaths, followed by COPD and ALRI infections (14%), and lung cancer (6%) (43).

Early daily time-series studies showed a strong correlation between daily PM levels and mortality in London and several United States of America (USA) cities (76-78). Following this work, an important study in six USA cities confirmed this association even when controlling for independent risk factors such as sex, age, cigarette smoking, comorbidities, occupation, body mass index (BMI) and educational background (79). Schwartz *et al.* found a stronger association between daily mortality and $PM_{2.5,}$ such as combustion-related particles, than with PM_{10} (80). A more recent meta-analysis confirmed the association between $PM_{2.5}$ and all-cause mortality: a 10μg/m^3 increase is associated with a 1.04% (95% Confidence Interval (CI) 0.52% - 1.56%) increased risk of death (81). As a result of $PM_{2.5}$ pollution, life expectancy in the European Union is estimated to be reduced by 8.6 months (43).

Respiratory disease

Chronic respiratory diseases, particularly obstructive airways disease such as asthma and COPD, and acute respiratory infections have been associated with ambient air pollution in ecological, observational and experimental studies, as described here.

Studies of children living nearby to areas with heavy traffic have shown a positive association between exposure to lorry traffic and symptoms of asthma and rhinitis, such as wheeze and persistent phlegm, as well as an increased occurrence of bronchitis, bronchiolitis and pneumonia and decreased lung function (82-84). Admissions to hospital for bronchitis and asthma are strongly associated with monthly ambient levels of PM_{10}, especially in children (85). Associations between daily levels of PM_{10} and decreased peak expiratory flow rate, symptoms of respiratory disease, and use of asthma medication have been shown (86, 87). Dockery *et al.* summarised multiple studies and concluded that with each 10μg/m^3 increase in PM_{10}, respiratory deaths, emergency department

visits due to respiratory disease, lower respiratory symptoms and asthma attacks increased by 3.4%, 1.0%, 3.0% and 3.0% respectively (88).

Studies of both healthy and asthmatic human subjects using exposure chambers have shown that airway hyper-responsiveness and airway resistance are increased following exposure to DEP (89-91). Changes in pulmonary function tests following exposure to traffic pollution were markedly worse in subjects with moderate asthma compared to mild, suggesting that DEP has a more damaging effect in humans with already compromised lung function (92).

Long term exposure to total levels of suspended particles have also been shown to increase symptoms of COPD and reduce lung function (forced vital capacity (FVC) and forced expiratory volume at one second (FEV_1)) in adults (93, 94). A UK cohort study of 16,000 patients found limited evidence for an association between ambient air pollution exposure and COPD incidence or COPD-related hospital admissions (95). A meta-analysis found no significant increased risk of COPD prevalence with PM exposure (pooled estimate from 3 studies; OR 1.11, 95% CI 0.93-1.31) but did find a significant association between COPD mortality and PM exposure (pooled estimate from 14 studies; OR 1.03, 95% CI 1.02-1.05) (96). However, some studies have found an association between COPD prevalence and PM exposure (97, 98). Given the chronicity of disease development in COPD, it is difficult to establish a causal relationship between ambient air pollution and COPD: further large studies with long-term follow up and carefully defined exposures are required to clarify the relationship (99).

A meta-analysis of 10 European birth cohorts estimated pollution exposure in over 16,000 children (aged 0-2 years) and found an increased risk of pneumonia with PM_{10} exposure (adjusted OR 1.76, CI 1.00-3.90, p=0.051) and nitrogen dioxide exposure (Odds Ratio (OR) 1.3, CI 1.02-1.65, p=0.024), but found no statistically significant association with $PM_{2.5}$ (100). It is unclear whether pre-natal or post-natal exposure is most crucial in childhood pneumonia risk, but a study in Poland demonstrated that pre-natal exposure to ambient $PM_{2.5}$ leads to reduced birth weight, and subsequent increased risk of bronchitis and pneumonia (101).

Cardiovascular and other diseases

There is strong epidemiological evidence for an association between ambient air pollution and cardiovascular disease. A meta-analysis found several air pollutants increased the risk of myocardial infarction (102). There are short-term associations between $PM_{2.5}$ levels and increased cardiovascular deaths and hospital admissions for heart rhythm disturbances, cerebrovascular events, ischaemic heart disease, peripheral vascular disease and heart failure have been found in a

nationwide study in the U.S., with the latter having the strongest association (103, 104). The increased cardiovascular risk may in part be explained by evidence for increased blood coaguability in healthy subjects following short term increases in ambient PM_{10} levels (105).

Epidemiological studies have also linked outdoor air pollution to Type 2 Diabetes Mellitus: air pollution exposure may increase the risk of developing Type 2 Diabetes Mellitus, and individuals with Type 2 Diabetes Mellitus may be more susceptible to the effects of air pollution (106). Several mechanisms for this have been proposed, including endothelial dysfunction, elevated systemic inflammation and cardiac autonomic nervous system dysfunction.

2.2.5.2. Health effects of occupational air pollution

Although occupational air pollution is not the focus of this thesis, it is briefly described here as it an important cause of respiratory disease that is distinct from other exposures in several ways; at risk population, pathologies and the economic and legal implications.

In industrialised countries, occupational asthma is thought to be the most common work-related respiratory disease (107) and occupational exposures are thought to account for approximately 15% of all adult asthma (108). Over 250 airway-sensitizing substances have been documented to cause allergic occupational asthma (109), yet due to the multi-factorial nature of asthma it can often be difficult to identify a specific causative agent.

Occupational exposures account for an estimated 14-15% of the population attributable fraction of COPD with a wide range of occupations implicated (108, 110), although studies are often confounded by cigarette smoke exposure.

Three dusts are mainly responsible for the global burden of pneumoconiosis: silica, asbestosis and coal dusts cause silicosis, asbestosis and coal workers' pneumoconiosis respectively. The burden of these diseases has declined in developed countries, due to regulation of industrial exposure, but pneumoconiosis remains an important occupational lung disease in many parts of the developing world. Unlike asthma and COPD, the pneumoconioses are essentially only caused by occupational exposures, and therefore the attributable fraction is assumed to be 100% (111). The Global Burden of Disease 2010 study estimated there were 125,000 deaths per year caused by pneumoconiosis worldwide (112).

There is also a large number of recognised occupational carcinogens (113). For example, asbestosis exposure is known to cause mesothelioma and lung cancer (114); pulp and paper mill workers are at an increased risk of lung cancer, lymphoma and leukaemia (115); metal compound inhalation is associated with lung cancer (116); workers exposed to wood-dust (for example, carpenters and

loggers) are at risk of upper and lower respiratory tract, gastrointestinal and haematological malignancies (117); and fire-fighters are exposed to a large number of airborne carcinogens, increasing their risk of haematological malignancies, and brain, bladder and lung cancers (118).

Exposure to airborne pollutants in the work-place is also associated with cardiovascular disease. A study of almost 250,000 Swedish construction workers found that exposure to any occupational particulate air pollution was associated with an increased risk of ischaemic heart disease (relative risk 1.13, 95% CI 1.07-1.19), with the highest risk being associated with exposure to diesel exhaust (relative risk 1.18, 95%CI 1.13-1.24) (119). However, no significantly increased risk of cerebrovascular disease was identified.

2.2.5.3. Health effects of household air pollution

Household air pollution is the eighth most important risk factor for global DALYs according to the Global Burden of Disease Study in 2015 (9). This is predominantly due to cardiovascular diseases, chronic respiratory diseases, respiratory infections and neoplasms. Global Burden of Disease study 2013 estimates nearly 3 million premature deaths a year are a result of household air pollution, but the WHO estimates this figure is closer to 4 million (51). Due to the lack of available data for the specific effects of household air pollution, morbidity and mortality estimates for Global Burden of Disease study 2013 are derived from an integrated exposure-response curve using data for ambient air pollution and tobacco smoke exposures (9). In this section, I outline the existing evidence for the association between household air pollution and several respiratory and cardiovascular outcomes.

Respiratory disease

Obstructive lung disease

Chronic Obstructive Pulmonary Disease

COPD is estimated to be the twelfth leading cause of years of life lost globally, causing 3 million deaths per year (120): 90% of these deaths occur in low income countries. The Burden of Obstructive Lung Disease (BOLD) Study found an overall moderate to severe COPD prevalence of 19% in Cape Town, South Africa (121). Data for the rest of sub-Saharan Africa are scarce (122), but more recent studies suggest that COPD prevalence of lower income countries in sub-Saharan Africa may be much lower: 3.6% of urban Malawians were found to have moderate to severe COPD (41).

Given the known association between cigarette smoke – a form of biomass - and COPD, it is unsurprising that there is evidence to support household air pollution as a cause of COPD. Two meta-analyses, both published in 2010, explored the association between household air pollution and COPD (123, 124). Kurmi *et al.* found 12 studies for inclusion, and concluded a positive

association between solid fuel use and COPD (OR 2.8, 95% CI 1.85-4.0). Hu *et al.* identified 15 studies, and reported a similar OR for developing COPD of 2.44 (95% CI 1.9-3.33), compared to those with no biomass smoke exposure. However, both of these meta-analyses are limited by the inclusion of studies with poorly defined exposures (*i.e.* no pollutant monitoring) and outcomes (*i.e.* lack of spirometric diagnosis of COPD). It is also of note that neither of these meta-analyses included any data from sub-Saharan Africa. A recent study from urban Malawi found no association between reported biomass use and obstructive spirometry, although direct pollutant monitoring was not performed (41).

Evidence for the impact of cessation of household air pollution exposure on lung function decline has not been consistent. A biogas intervention and a ventilation intervention in China reduced annual FEV_1 decline by 12% and 13% respectively over a 9 year follow up period, but these interventions were not randomly allocated (125). In the context of randomised control trials (RCT) of cookstove interventions, two studies identified a reduction in respiratory symptoms in women, but had conflicting findings regarding lung function decline. An RCT in Mexico found a reduction in lung function decline (31ml vs 62 ml over 1 year, p=0.01) (126), whereas the RCT in Guatemala found no effect on lung function (127). Both these studies had short follow up periods and relatively young populations, which may have limited the ability to evaluate the effects on a chronic disease.

Asthma

330 million people worldwide have asthma (15), resulting in 400,000 deaths per year (16). A systematic review found that prevalence rates vary widely across sub-Saharan Africa, from 4 to 20%, with the highest prevalence being found in 'westernised' and urban areas (128, 129). However, most of the available data relates to children, with only 3 surveys in adults identified.

Household air pollution is not only a trigger for asthma exacerbations, but exposure may also increase asthma prevalence (130). In children aged 6-7 years and 13-14 years, the International Study of Asthma and Allergies in Children (ISAAC) found that sole use of an open fire for cooking was associated with an increase in reported wheeze in the past year (OR 2.17, 95% CI 1.64-2.87 and OR 1.35, 95% CI 1.11-1.64 respectively).

Bronchiectasis

There is an absence of data on the prevalence of bronchiectasis in sub-Saharan Africa (in part due to the requirements for a radiological diagnosis) and no epidemiological evidence available for an association between household air pollution and bronchiectasis. However, chronic cough is common in countries where household air pollution is highly prevalent, and its pathology and underlying aetiology have not been described in detail (2). An association between biomass exposure and

sputum expectoration has been reported in Malawi (OR 7.05, 95% CI 2.28-21.80) (41). In an autopsy study of Mexican women with COPD, two thirds of women with exposure to biomass smoke but no history of cigarette smoke had evidence of bronchiectasis (131).

Restrictive lung disease

In the aforementioned spirometric study in urban Malawian adults, restrictive abnormalities were more common than obstructive abnormalities across all gender and age strata (41). Using the Third National Health and Nutrition Examination Survey (NHANES III) reference ranges, the prevalence of spirometric restriction was 38.6% (SE 2.1), although this was much lower using locally derived reference ranges (9%, SE 1.2). The majority of individuals (85%) reported biomass exposure for more than 6 months, however no association between biomass exposure and restrictive disease was found.

Respiratory cancers

Emissions from burning coal and solid fuels are classified as known (Group 1) and probable (Group 2A) carcinogens, respectively, according to the International Agency for Research in Cancer (132).

A meta-analysis, including 11 mainly small hospital-based studies, detected an OR of 1.70 (95% CI 1.45-2.00) for the risk of nasopharyngeal cancer associated with exposure to household air pollution, increasing to OR 3.18 (95% CI 2.36-4.3, n=6) following adjustment for tobacco smoking (2). However, all of these studies relied on self-reported exposures, and none were conducted in sub-Saharan Africa.

Meta-analysis has detected a pooled OR of 2.31 (95% CI 1.94-2.76) for the risk of lung cancer in relation to exposure to household air pollution (2). A higher carcinogenic risk has been identified with burning coal (OR 1.82, 95% CI 1.6-2.06) compared to biomass fuels (OR 1.50, 95% CI 1.17-1.94) (133). Risk is greater in females than in males (p=0.034), and the pooled risks differ according to cancer histological subtype (squamous cell carcinoma: OR 3.58, 95% CI 1.58-8.12; adenocarcinoma: OR 2.33, 95% CI 1.72-3.17). The majority of studies were conducted in China, where coal is the predominant fuel.

Tuberculosis

A systematic review published in 2013 identified 13 studies (including 10 case-control studies) examining the relationship between household air pollution and tuberculosis (134). These 13 studies had varied results, with only three studies reporting a statistically significant association between household air pollution and tuberculosis. Meta-analysis of the 10 case-control studies identified a pooled overall OR of 1.30 (95%CI 1.04-1.62. p=0.019), with an increased effect size when the analysis

was limited to female participants only (7 studies, OR 1.7, 95% CI 1.00-2.89). However, methodological limitations in the quality of studies were identified, with risk of exposure misclassification (no studies objectively measured pollution exposure), lack of adjustment for potential confounders (*e.g*. tobacco smoking) and potential selection bias (use of hospital controls). The underlying mechanisms which may lead to an increased risk of tuberculosis are not fully understood. Impaired phagocytosis of *Mycobacterium tuberculosis* has been demonstrated in human alveolar macrophages exposed *in vitro* to respirable-sized particulates, but there was no overall effect on killing of *M. tuberculosis* (135). In bronchoalveolar lavage (BAL) samples obtained from Malawian adults with tuberculosis, alveolar macrophages that were infected with *M. tuberculosis* and uninfected alveolar macrophages were found to be contain high levels of particulate matter (presumed from exposure to air pollution) (136). However, the phagocytic capacity of the alveolar macrophages appeared to be unaffected by *M. tuberculosis* infection, HIV infection or degree of particulate matter loading (136).

Pneumonia

There is experimental evidence from animals and humans which may explain an increased risk of respiratory infection from exposure to household air pollution. Experimental installation of intranasal ultrafine particles in mice has been shown to not increase susceptibility to pneumococcal infection (137). Other animal models have demonstrated an up-regulation of pro-inflammatory mediators, including interleukin 6, in response to particulate exposure (138, 139). An *in vitro* model of exposure of human alveolar macrophages respirable particulates, which was demonstrated to be morphologically comparable to *in vivo* exposure to household air pollution, demonstrated increased cytokine release and altered macrophage function (135). Phagocytosis of pneumococci was impaired, and alveolar macrophage oxidative burst was reduced, providing insight into potential mechanisms for increased susceptibility to bacterial pathogens following exposure to household air pollution. Reduction in oxidative burst, along with reduced cytokine induction, has been demonstrated in alveolar macrophages obtained by BAL from Malawian adults chronically exposed to household air pollution from cooking, although phagocytosis and proteolytic functions were unaffected (140). Furthermore, exposure to household air pollution has been shown to alter the lung microbiome of healthy Malawian adults, with an increased abundance of potentially pathogenic bacteria (141). These findings may explain the association between household air pollution and pneumonia that has been described in some epidemiological studies.

Meta-analyses of studies examining the relationship between household air pollution and ALRI in children have found a significant association: Dherani *et al.* reported a pooled OR of 1.78 (95%CI 1.45-2.18) and Po *et al.* reported a pooled OR of 3.53 (95%CI 1.93-6.43) (12, 142). Half a million

deaths per year are estimated to be caused by childhood ALRI resulting from household air pollution exposure (51). An RCT of a woodstove with a chimney (compared to a traditional open fire) in Guatemala (the RESPIRE trial) found that a 50% reduction in CO exposure significantly reduced physician-diagnosed pneumonia in children under the age of 5 (rate ratio 0.82, 95%CI 0.70-0.98), but risk reduction was not significant in the intention-to-treat analysis (rate ratio 0.84, 95% CI 0.63-1.13) due to substantial exposure overlap between the two groups. A recent RCT of an improved cookstove in rural Malawi, with almost 16000 years of follow-up, did not find a reduction in pneumonia in children under the age of 5 (incidence rate ratio 1.01, 95% CI 0.91-1.13) (143).

Given the epidemiological evidence in children, supported by laboratory data providing likely mechanistic pathways, it is plausible that an association between household air pollution and pneumonia in adults exists. The epidemiological evidence for this is explored in detail in chapter 3.

Cardiovascular disease

The mechanistic effects of household air pollution on the cardiovascular system have not been extensively studied, and it is thought that due to the differences in pollution composition, the effects may differ from those of ambient air pollution data (144). Laboratory and observational studies have shown that biomass smoke exposure can cause arterial stiffness, decreased heart rate variability and raised inflammatory cytokines (145, 146). Chronic exposure to biomass smoke has been shown to be associated with increased carotid intima media thickness and atherosclerotic plaques (147), both associated with cardiovascular events (148).

Exposure to household air pollution has been associated with elevations in blood pressure (149) as well as increased prevalence of hypertension (150, 151). A cleaner burning cookstove intervention in Nicaragua significantly reduced systolic blood pressure in older women (mean change -5.9mmHg, 95% CI -11.3 - -0.4, p=0.05), but not in other sub-groups (152).

Nested within the RESPIRE trial, between-group and before-and-after comparisons were used to demonstrate a statistically significant reduction in the occurrence of ST-segment depression on electrocardiogram (OR 0.26, 95% CI 0.08-0.90 in between-group comparisons), although no significant change in heart rate variability was found (153). These changes in electrocardial activity suggest that household air pollution may affect ventricular repolarisation.

A systematic review of household air pollution and coronary heart disease concluded that the evidence base is limited, but that current epidemiological evidences suggests an increased risk of coronary heart disease from household air pollution (154). A large cohort study from Iran found an increase in ischaemic heart disease mortality (10-year hazard ratio 1.14, 95% CI 1.06-1.21) (155).

The same Iranian cohort demonstrated a non-significant increased risk of stroke mortality associated with exposure to household air pollution: 10-year hazard ratio 1.08, 95% CI 0.99-1.17 (155). A large cross-sectional study from China detected an increased risk of stroke (OR 1.87, 95% CI 1.03-3.38) in adults reporting household air pollution exposure (151). Further prospective studies using air pollutant monitor to confirm exposure classification are required to confirm these findings.

2.2.6. Measuring air pollution exposure

Assessing an individual's exposure to air pollution is very complex and, to date, there is no gold standard method. Studies examining the health impacts of air pollution exposure have used a wide variety of methods. Crude assessments, such as questionnaires of participant reported exposure, are limited by inaccurate estimates and recall bias, and fail to account for the many different variables that will affect levels of pollution, such as differences in cooking practices, fuel type, fuel moisture content, combustion conditions in the stove and ventilation. Measuring indoor levels, ambient levels or personal exposure to specific pollutants provides quantifiable data but these methods are not without their limitations, as discussed below.

It is not yet clear, from the available data, which components of household air pollution are the most harmful to health and should be monitored. Studies that have failed to demonstrated an association between one specific component of household air pollution, such as PM_{10}, and disease may be misleading as an alternative component of household air pollution may be associated with that disease.

With current available methodology, the most representative exposure assessment is likely to be achieved by individual monitoring for a variety of pollutant constituents, to evaluate the combined effects of the different pollutants on health. The resource constraints for many studies limit this.

Inaccurate assessments of exposure leads to biased estimates of the relationship between air pollution exposure and health. It is essential to obtain accurate, quantitative emission-specific assessments in order to demonstrate the true burden of disease associated with air pollution. More detailed information regarding the effects of specific air pollution components will facilitate assessment of air pollution interventions, allowing resources and policies to be directed accordingly.

2.2.6.1. Particulate matter monitoring

PM monitoring has long been a component of interest in outdoor air pollution studies, and has also been used in household air pollution studies (79, 156-158). High PM exposure has been shown to be associated with cardiovascular disease, poor neonatal outcomes, lung cancer, respiratory infections in children and increased mortality (102, 158-161).

Gravimetric methods

Gravimetric devices use an air pump to draw in a sample of air through a filter, and the PM is collected onto the filter. Depending on the pore-size of the filter, different types of PM can be collected, for example, $PM_{2.5}$ or PM_{10}. The filter, which has been pre-weighed prior to sampling, is then weighed again using an electronic microbalance under laboratory conditions to quantify the mass of PM. In addition, the collected PM can be biochemically analysed to determine the constituent components.

Traditional gravimetric sampling methods are best suited to static monitoring, rather than ambulatory monitoring, due to the size and weight of the devices, as well as the noise generated by the air pump. Other challenges include the expertise required for using the devices in the field (as mishandling of the sensitive filters can destroy the collected sample), and the expense of transporting and analysing the filters under laboratory conditions. Electrostatic effects, sorption of moisture and volatiles, and temperature must all be carefully controlled to allow accurate gravimetric analysis (162). Devices which are solely gravimetric can only provide data on the total amount of PM exposure over the sampling period, and do not provide real-time measurements in the fluctuations of exposure over time. However, newer designs, such as the RTI MicroPEM (www.rti.org) and the TSI DustTRAK DRX Aerosol Monitor 8533 (www.tsi.com), which combine photometric monitoring with gravimetric filters for calibration in lightweight devices are overcoming many of these limitations (Figure 2-2).

Photometric methods

Photometric devices measure PM concentrations using diffraction of light, providing real-time data for exposure patterns. Multiple devices are now available including the University of California, Berkeley Particle and Temperature Sensor (UCB-PATS) (www.berkeleyair.com), a modified smoke alarm which uses a photoelectric detector to measure $PM_{2.5}$ concentrations as low as 25$\mu g/m^3$ (Figure 2-2). Although lightweight and small, the UCB-PATS is not designed to be moved around during monitoring, so is best suited for static household monitoring. Devices suitable for ambulatory monitoring include the TSI SidePak AM510 (www.tsi.com), which actively samples the air using a pump and measure the refraction of a laser beam caused by $PM_{<0.1}$, $PM_{2.5}$ or PM_{10}; the ThermoFisher Scientific *personal* DataRAM pDR-1000AN Monitor (www.thermofisher.com) which uses single beam nephelometry to detect PM_{10}; or the Aprovecho Indoor Air Pollution Monitor (www.aprovecho.org) which uses fan-assisted air sampling and combines red laser scattering photometer $PM_{2.5}$ detection in the range of 0-60,000 $\mu g/m^3$ with electrochemical cell CO detection in the range of 0-1000 parts per million (ppm) (Figure 2-2).

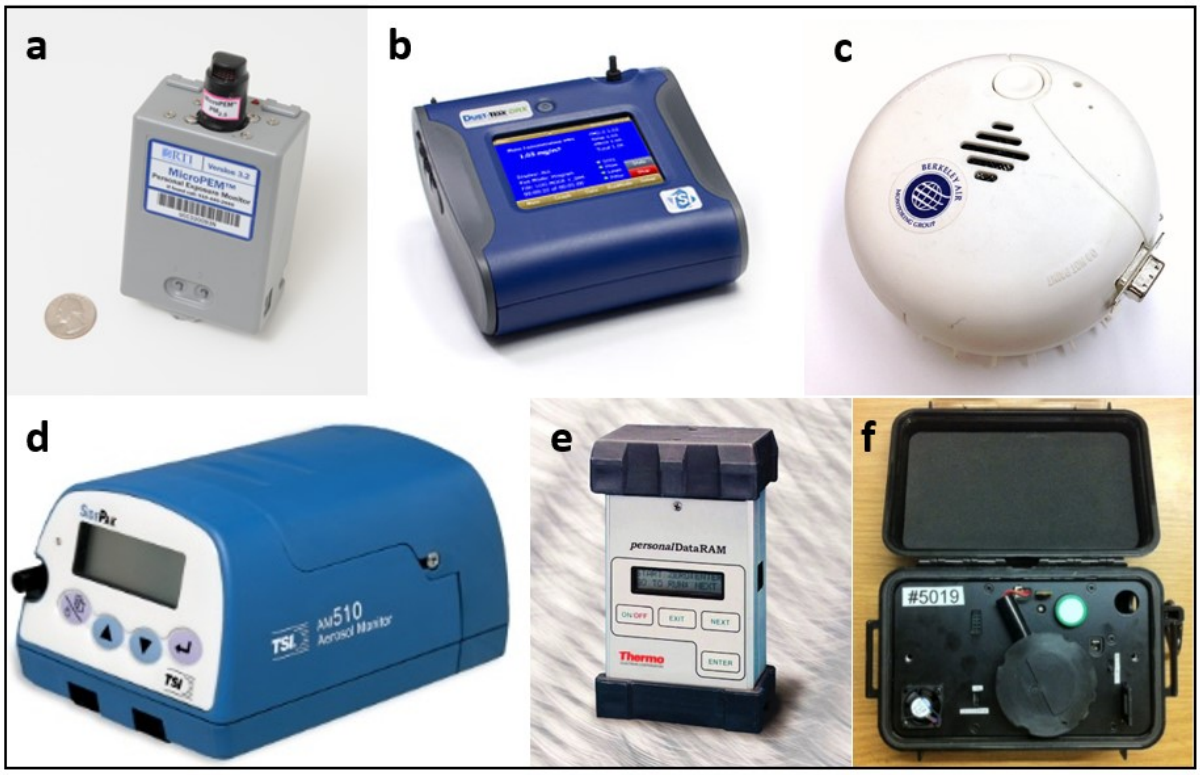

Figure 2-2: Devices used for particulate matter air pollution monitoring.
a) RTI MicroPEM (www.rti.org); b) TSI DustTrak DRX Aerosol Monitor 8533 (www.tsi.com); c) University of California, Berkeley Particle and Temperature Sensor (UCB-PATS) (www.berkeleyair.com); d) TSI SidePak AM510 (www.tsi.com); e) ThermoFisher Scientific *personal* DataRAM pDR-1000AN Monitor (www.thermofisher.com); f) Aprovecho Indoor Air Pollution Monitor (www. aprovecho.org).

2.2.6.2. Carbon Monoxide monitoring

CO monitoring is particularly useful where there is an absence of high $PM_{2.5}$ levels, for example, with charcoal burning. CO monitoring has been used in previous household air pollution studies, and has been shown to be associated with pneumonia and poor neurodevelopmental outcomes in children (163, 164). Time-weighted average exposures to CO can be measured using passive diffusion tubes, such as Gastec CO tubes (Gastec Corp, Kanagawa, Japan) (Figure 2-3), in which a brown stain appears in the tube as a result of a chemical reaction in which CO reduces sodium palladosulfite to liberate metallic palladium; the length of the stain relates to the cumulative dose of CO (165). Ambulatory, real-time exposure measurements can be collected using an Aprovecho Indoor Air Pollution Monitor (discussed above) or a very lightweight, portable Lascar EL-USB-CO300 Carbon Monoxide USB Data Logger (www.lascarelectronics.com), which can detect CO in the range of 0-300ppm (Figure 2-3).

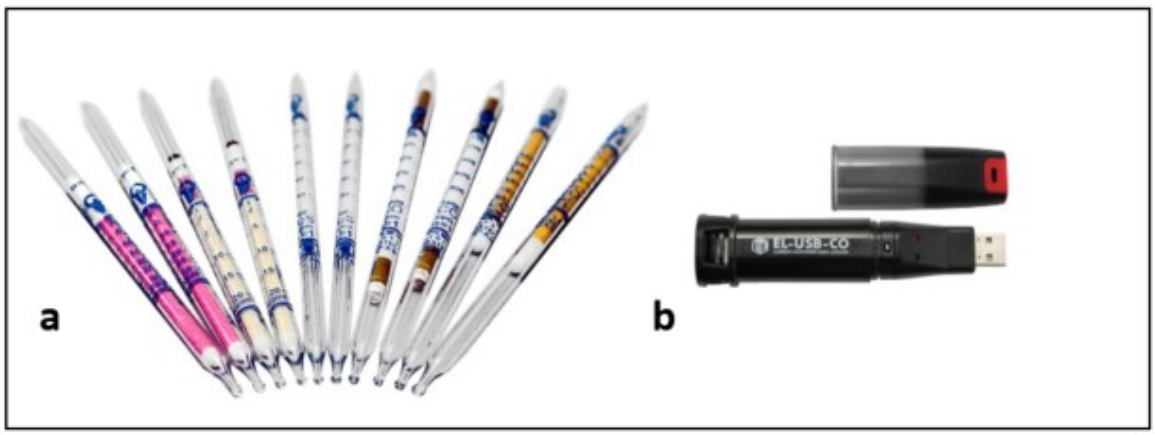

Figure 2-3: Devices used for carbon monoxide air pollution monitoring.
a) Gastec CO tubes (Gastec Corp, Japan); b) Lascar EL-USB-CO300 Carbon Monoxide USB Data Logger (www.lascarelectronics.com).

2.2.6.3. Placement of air pollution monitors

Location of measurement

Measuring household air pollution exposure is usually conducted with static household monitors, often placed in the kitchen where the highest levels of pollution are likely to be detected, or with ambulatory personal monitors which are carried by or attached to the participant.

Static monitoring fails to consider how an individual's behaviour may affect their exposure, but can be useful if the emissions from a particular source are of interest (*e.g*. if an improved cookstove is being tested). Studies which combine household air sampling with time-activity diaries or global positioning system tracking of individuals go some way to address this, but as pollutant levels vary greatly even between different parts of the same room, their accuracy is be limited. For this reason, placement of the monitors should be standardised across study participants. In several studies, the monitor is placed 1m away from the cooking fire and 1m off the ground to sample the air space of people who are using or sitting by the fire, without damaging the monitor by placing it too close to the heat source.

Personal exposure monitoring - using devices attached to a participant's clothes or carried by the participant - may provide more accuracy in terms of an individual's overall exposure as it can account for both indoor and outdoor exposures, as well as behavioural factors (2). However, personal monitoring does not truly reflect their exposure which is dependent on their respiratory rate and tidal volume. Many studies have been limited to only measuring one or two pollutants, such as CO or PM_{10}, in this way and it can be burdensome for the participant. The position of the device on the person must also be considered – ideally the air sampling component of the monitor should

be placed close to their breathing space (*i.e.* near to their face) and should be standardised across participants.

Timing of measurement

The timing and length of pollution monitoring needs to be carefully considered as the methodology used will influence the findings. If short-term exposures are of interest, then one-off short-term monitoring may be acceptable, although variations in behaviour and exposure over different days will not be accounted for. If chronic air pollution exposure is of interest, repeated measurements over time will improve reliability of results, and may help to account for seasonal changes in exposure. Practical and resource limitations, as well as the burden to participants, must also be considered. Studies are required to find a compromise between continuous long-term monitoring and the financial cost of this. Cynthia, A.A. *et al.* demonstrated no substantial difference in results obtained in a 48 hour period to a 96 hour period, and argued that the difference observed could be accounted for by a small increase in sample size (156).

It is not established whether average exposures, peak exposures or time exposed to pollutants above a certain threshold level correlate best with health outcomes (2). This may vary depending on the pollutant and the health outcome of interest. Detailed real-time exposure measurements of a variety of pollutants may help to clarify this.

2.2.6.4. Biomarkers of air pollution exposure

An alternative approach to directly monitoring environmental exposures is to test for the presence of a substance in human samples, such as blood, urine or sputum samples, which indicates that individual has had a particular environmental exposure. Several biomarkers have been identified as potential surrogate markers for exposure to household air pollution, but all have limitations (166-170).

A biomarker to be used for exposure monitoring and intervention purposes, should be closely associated with exposure, be adequately sensitive and specific, have strong correlations with currently used methods for measuring exposure, reflect relatively short-term fluctuations in exposures, be consistent across heterogeneous populations, be cost-efficient, have sampling methods that are acceptable to the user population, and be feasible for use in the field (ideally point-of-care testing or be stable to temperature, storage and transport conditions)(170).

Airway macrophage particulate load (AMPL) has been demonstrated to be a potential biomarker of household air pollution exposure, as increased AMPL has been detected in individuals who report exposure to household air pollution compared to those who do not (171). Furthermore, AMPL has

been shown to be significantly different in individuals who use different types of fuel for cooking and lighting (65). However, to date, this has not been correlated with careful exposure monitoring. The feasibility of measuring AMPL is discussed in Chapter 4.

Exhaled carbon monoxide (eCO) has also been proposed as a potential biomarker of household air pollution exposure, which will be discussed in more detail in Chapters 5 and 6.

Epithelial/endothelial activation, damage, or death and increased oxidative stress are well recognised as playing a role in the early pathophysiology of COPD resulting from cigarette smoke exposure (172), and similar processes may play a role in air pollution induced lung damage; increased oxidative stress and antioxidant up-regulation has been identified in household air pollution exposed individuals in Malawi (173). Identification of a mediator of these pathophysiological processes which correlates with air pollution exposure will not only provide a useful tool for exposure assessment but will also provide insight into mechanisms of pulmonary damage that occur. Exposure or response molecules that potentially meet the criteria for biomarkers discussed above include those in

Table 2-2. However, a limitation of using downstream markers of tissue damage is that they are not specific to lung damage, and not limited to the effects of inhaled exposures.

Degradation products from biomass combustion, such as levoglucosan, methoxyphenols, and metabolites of hydroxylated polycyclic aromatic hydrocarbons, can be detected in urine and are increased following wood smoke exposure (166, 169, 174-176). However, results have not been consistent and the utility of these biomarkers is limited by the fact that these compounds are not specific to biomass combustion, and methoxyphenols and levoglucosan can be ingested in food products (168, 170).

Identification of an appropriate biomarker could obviate the need for costly and intensive exposure monitoring. Furthermore, a biomarker will account for differences in exposure caused by behavioural factors and tidal volumes that external monitoring cannot assess. Further exploration of potential biomarkers is warranted, with careful characterisation of their association with exposure.

Table 2-2: Pathophysiological molecules – potential biomarkers of household air pollution exposure.

Pathophysiological effect of pollution exposure	Potential biomarker	Potential methods of detection	Previous applications
Endothelial activation, damage and death	Ceramide, a pro-apoptotic sphingolipid implicated in alveolar cell apoptosis and increased oxidative stress (177)	Liquid chromatography / tandem mass spectrometry, Western blotting, ELISA	Detected in rat urine in hepatotoxicity (178) Detected in human urine in Farber disease (179)
	RTP801, a stress induced molecule implicated in alveolar cell apoptosis and increased oxidative stress		Detected in urine
	EMAP II, a monocyte- and epithelial- secreted cytokine stimulated by smoke exposure and hypoxic stress that induces cell apoptosis and inflammation (180)		Detected in serum
Epithelial damage	RAGE (181)	ELISA	Detected in induced sputum in asthma (182, 183)
	Surfactant protein D		Detected in induced sputum in asthma (184) Detected in serum in wood smoke exposure (185)
Collagen degradation	PGP, a collagen degradation product which amplifies smoke induced inflammation	Liquid chromatography / mass spectrometry	Detected in induced sputum in COPD (186, 187)
Oxidative stress and damage	MDA, a lipid peroxidation marker	ELISA, liquid chromatography, radioimmunoassay	Detected in serum, urine, sputum and exhaled breath in humans exposed to air pollution, patients with COPD, nephropathy and those exposed to arsenic (188-191)
	8-isporostane		Detected in urine of humans exposed to wood smoke (192) Detected in sputum and exhaled breath in COPD and asthma (193, 194)
	8-oxodG, indicates oxidative induced DNA damage		Detected in urine in nephropathy (190) Detected in induced sputum of healthy children (195)

ELISA: enzyme-linked immunosorbent assay; EMAP II: endothelial monocyte-activating polypeptide-II; RAGE: r**eceptor for** a**dvanced** g**lycation** e**nd** p**roducts;** PGP: p**roline-glycine-proline peptide**; COPD: chronic obstructive pulmonary disease; MDA: m**alondialdehyde**; 8-oxodG: **8-oxo-7,8-dihydro-2'-deoxyguanosine**.

2.2.7. Reducing exposure to air pollution

To address the health impacts of air pollution exposure there is growing international recognition of the need to reduce exposure to air pollution: multiple strategies aim to achieve this.

2.2.7.1. Reducing ambient air pollution exposure

WHO Air Quality Guidelines were first produced in 1987 with the aim of reducing the health impacts of air pollution (196). Specific targets for selected air pollutants are given, to assist countries in implementing their own standards, pollution monitoring programmes and pollution reduction policies. Such policies vary from country to country, according to economic, political and social factors as well as feasibility of implementation, but may include legislation for industrial and transport related pollution.

2.2.7.2. Reducing household air pollution exposure

Improved cookstoves

In response to growing concerns about household air pollution, The Global Alliance for Clean Cookstoves (www.cleancookstoves.org) - a public-private initiative hosted by the UN Foundation - aims to address the public health impact of household air pollution. One aspect of their strategy is to have clean, efficient cooking solutions in 100 million homes by 2020. This will have wide-reaching economic, social and environmental impacts, but to ensure that investment in intervention programmes is appropriately directed from a public health perspective, the true burden of disease needs to be clarified (197, 198).

Improved cookstove is an umbrella term used to describe a wide variety of stove designs which improve the efficiency of fuel combustion compared to a traditional open 3-stone fire (see Figure 2-4). Not all improved cookstoves reduce emissions enough to have a measurable health benefit, but may provide other benefits for the user. Improved combustion efficiency means that less fuel is required to generate the same amount of energy: in fuel-scarce environments, this can save the user a large amount of time (as less fuel needs to be gathered) or money. As woman and children bear responsibility for fuel collection in many societies, reducing the time spent doing this can create time for education or income generating activities. In some settings, women and children are vulnerable to physical attacks when collecting firewood in rural areas: reducing fuel consumption can reduce the risk of this happening.

Figure 2-4: Examples of types of improved cookstoves
a) Chitezo Mbaula stove; b) Plancha stove (image reproduced from http://aprovecho.org/newsletters/); c) Rocket stove (StoveTec Rocket Cascadia Cookstove 1-door, image reproduced from https://theepicenter.com/stovetes-cascadia-biomass-cookstove.html); d) Gasifier stove (African Clean Energy, ACE-1 Ultra-Clean Biomass Cookstove, image reproduced from www.catalog.cleancookstoves.org/stoves/268); e) Fan assisted gasifier stove (Philips Cookstove, image reproduced from https://www.changemakers.com/discussions/entries/dazin-renewable-cooking-fuel-all).

One of the lowest cost stoves available are simple portable clay stoves for burning biomass fuels, known in Malawi as Chitetezo Mbaula, which translates to “protecting cookstove”. The benefits of these are that they reduce fuel consumption, and can be made locally using local materials and limited expertise: a local market can therefore be established, generating employment. However, there is no evidence to support claims that there is large enough emission reduction to have a health benefit. Larger, immobile stoves, which have a stone or concrete combustion chamber which can be made locally, come in a variety of designs and can be fitted with a “plancha” (metal stove top) and chimney, to reduce household air pollution. A disadvantage it that the lack of portability means that the stoves cannot be moved for cooking outside during dry weather, so may lead to higher levels of pollution within the home if a chimney is not fitted or maintained. Portable rocket stoves have a small combustion chamber containing an insulated vertical chimney. This ensures almost complete combustion before the flame reaches the cooking surface, and have been demonstrated to reduce fuel consumption and emissions compared to a 3-stone fire. However, the metal double-skinned

chamber and insulation can often not be sourced locally, increasing the cost of these stoves. Biomass burning stoves which have the greatest impact on emissions, also come at the highest financial cost.

Gasifying technology provides a cleaner solution, whilst still using widely available biomass fuels. 'Gasification' describes the process of converting solid fuel into a gaseous fuel. If this is done in a controlled manner, in a biomass gasifier, rather than simultaneously with all other stages of combustion, as occurs in an open fire, then energy is generated with far fewer emissions (199). Natural-draft gasifier stoves are a relatively simple way of achieving this, and can often be made using locally sourced materials such as scrap metal sheets. Many gasifier stoves also have the capabilities to produce charcoal, and the heat created in the process is used for cooking. Fan assisted gasifier stoves, such as the Philips cookstove (www.philips.com) which comes with a solar powered fan to assist the air draw through the combustion chamber, create a cleaner burn. This can reduce emissions by up to 90% (200), but the high cost means this stove is currently unaffordable for many who are reliant on solid fuel use.

Fuel diversification

Alternatively, where available, diversification of fuel type away from solid fuels can reduce household air pollution. By 2040, access to electricity is projected to have improved thanks to growth in renewable energy resources, but more than half a billion people – mainly in rural areas – will remain without electricity (201).

Domestic biogas production is increasingly popular in the Indian Subcontinent: dairy manure is anaerobically digested in airtight circular pits outside the home to produce biogas, which is then piped directly to the kitchen for cooking. Where livestock are readily available, this can be an affordable and sustainable method for generating domestic energy.

Although more expensive, liquid petroleum gas, which predominantly consists of propane and butane, is a less polluting fuel at the point of use that is a realistic option in many middle-income countries in Asia or Latin America. Solar power and hydro-power are further examples of sustainable clean energy, but lack of infrastructure and high costs make widespread use challenging.

Even where cleaner fuels are available, 'fuel stacking' prevents households achieving very low levels of household air pollution. 'Fuel stacking' is the concept of a household using multiple fuel types for their energy needs. Use of multiple fuel types and technologies is the norm in many settings, as one cooking method is rarely suitable for all types of food consumed (2). Fuel use depends on the current availability and cost, cultural norms, as well as what the fuel is required for, as fires often

fulfil several functions rather than just cooking (202). As household income increases, the number of types of fuel that are used increases, rather than a complete switch to an alternative fuel (203). This can have important policy implications when trying to address household air pollution, as income is not the sole factor to be considered.

Ventilation

As mentioned above, an important strategy to reduce household air pollution is to improve ventilation in the cooking area. This can be achieved by installation of chimney stoves or smoke hoods, by ensuring kitchens have opening windows or simply by cooking outside when the weather allows. Chimney stoves can theoretically cause significant reductions in pollution, as the smoke is contained within an enclosed system. However, poorly designed or poorly maintained chimneys can lead to inefficient combustion resulting in higher emissions. Smoke hoods, which simply sit over the stove or fireplace, provide a simpler alternative and can reduce household air pollution by up to 80% in field testing (204). A disadvantage of relying on ventilation to reduce indoor exposures is that the pollutants are simply diverted to the outdoor environment (163, 197): domestic use of solid fuels is thought to be an important contributor to ambient pollution levels, particularly in urban areas. Conversely, ambient air pollution may move from the outdoor environment into the household; this infiltration is determined by house air exchange rates (2). This movement of pollution between the two environments gives rise to several challenges: monitoring of exposures must take a variety of sources into consideration; apportioning causality of health effects to either household or ambient pollution is complex; and interventions to reduce exposure must take this movement of pollution (and its impact on neighbouring households, for example) into consideration.

Behaviour

Interventions to impact the behavioural aspects of exposure, as discussed in section 2.2.3.2., may also help but there is currently little evidence to support a health benefit from such interventions.

2.2.8. Household air pollution in Malawi

A comparison of rural and urban areas in Malawi in 2008 found rural homes tend to use firewood and therefore have high PM levels, whereas urban homes tend to use charcoal and so have high CO levels (3). Whilst 100% of households reported using wood as their main fuel for cooking in rural areas, only 21% of households in urban areas did. Charcoal was reported as the main fuel type by 66% of urban households, whilst 6% reported using both wood and charcoal, and 9% used electricity. Kerosene use for lighting was also more common in rural areas compared to urban areas (87% vs 61%), where 29% of households had electricity for lighting, compared to only 3% in rural areas. Further differences were noted in cooking locations: only 6% of rural households reported

cooking inside the main house during dry season, whereas 52% of urban households cooked inside the main house.

Household air sampling in both the rural and urban areas revealed pollution levels in excess of WHO air quality guidelines in all homes sampled in the above mentioned study from Malawi (3). The WHO Air Quality Guidelines recommend that 24-hour mean exposures of $PM_{2.5}$ should not exceed 25 $\mu g/m^3$ (196). Using UCB-PATS devices (see section 2.2.6.1.), in the above study, the mean $PM_{2.5}$ time weighted average for all homes was 204$\mu g/m^3$, with no significant difference detected between rural and urban homes. However, 52% vs 17% of homes had $PM_{2.5}$ levels in excess of 250$\mu g/m^3$ for more than 1 hour per day in rural and urban areas respectively ($p = 0.112$). There was no significant difference in transition metal content of gravimetric PM samples when compared by fuel type. Urban homes had significantly higher levels of CO than rural homes (mean CO time weighted average 6.14 ppm vs 1.87 ppm, $p<0.001$), and this was associated with charcoal use ($p<0.001$) (3).

2.3. Literature review: Risk factors for pneumonia

2.3.1. Pneumonia

2.3.1.1. Definition and classification

Pneumonia is inflammation of the alveoli, with associated accumulation of inflammatory exudate resulting in pulmonary consolidation, often caused by infectious agents. Pneumonia usually presents with productive cough, shortness of breath, pleuritic chest pain and fever, and may lead to hypoxia and systemic compromise if severe.

ALRI is a term that encompasses a broad range of diagnoses - including pneumonia, acute bronchitis and bronchiolitis - that, unlike upper respiratory infections, affect the lower respiratory tract, below the level of the epiglottis. Infections of the respiratory tract that have a natural history that persists longer than 2 weeks, such as tuberculosis, are not usually referred to as ALRI.

Pneumonia can be classified by its aetiology, or by the anatomical location of affected lung. Community-acquired pneumonia – the subject of this thesis – refers to pneumonia caused by infection acquired outside of healthcare facilities, and therefore the onset is prior to admission to or within 48 hours of admission to a healthcare facility. Healthcare-associated or hospital-acquired pneumonia refers to pneumonia caused by organisms acquired in healthcare facilities (onset 48 hours or more after admission), including hospitals and nursing homes, and is more likely to be caused by multi-drug resistant organisms. Ventilator-associated pneumonia is that which is

contracted whilst a patient is receiving mechanical ventilatory support, arising more than 48 hours after endotracheal intubation.

Lobar pneumonia refers to infection of a single lobe of the lung, or multi-lobar pneumonia describes the consolidation in multiple lobes which is often more severe. Bronchopneumonia affects the bronchi and bronchioles, whereas interstitial pneumonia is the inflammation of the pulmonary interstitium which can be caused by viruses or atypical bacteria.

Expert bodies and research studies have used a variety of definitions for pneumonia (see

Table 2-3). It is of note that the only study listed here to restrict their definition by symptom duration (to 14 days or less) is the study conducted in Kenya by Scott *et al.* where tuberculosis and HIV prevalence are high (6). This may be because the authors of this study felt it was important to exclude individuals with tuberculosis, whereas this may have been less of a priority in other settings where the burden of tuberculosis is lower.

2.3.1.2. Pathogenesis

Bacterial or viral pneumonia are most common, but other microorganisms – including fungi – and non-infectious insults may also be responsible. Microbiological aetiology varies across different regions globally (6, 205-207), but *Streptococcus pneumoniae* – a Gram positive, facultative anaerobic bacterium – is recognised as being the most common cause of bacterial pneumonia worldwide (6, 208, 209). The diagnosis can be complicated by tuberculosis, which may present as an acute pneumonic illness (6, 207), or by opportunistic infections in susceptible patients (210, 211), particularly in settings where tuberculosis and HIV infection prevalence are high.

2.3.1.3. Diagnosis

Diagnosis of pneumonia is usually based on clinical assessment of history and examination of signs, supported by radiological evidence of pulmonary consolidation, usually by chest radiograph (see

Table 2-3). Distinguishing pneumonia from other forms of ALRI, such as milder, self-limiting viral infections which cause acute bronchitis, using clinical signs alone can be challenging. No individual sign or symptom is useful for making this distinction. Pneumonia is defined as involving consolidation of the lung parenchyma, however, a prospective study of individuals treated with antibiotics for ALRI with new focal chest signs found that only 39% of individuals had chest x-ray changes (212). Early chest radiograph may fail to identify parenchymal opacifications which develop over subsequent days (213).

Table 2-3: Definitions of pneumonia

Expert body / Research study	Diagnosis	Definition
British Thoracic Society, 2009 (214)	*Community Acquired Pneumonia*	Symptoms and signs consistent with an acute lower respiratory tract infection associated with new radiographic shadowing for which there is no other explanation.
European Respiratory Society, 2011 (215)	*Suspected Community Acquired Pneumonia*	An acute illness with cough and at least one of new focal chest signs, fever >4 days or dyspnoea/tachypnoea, and without other obvious cause.
	Definite Community Acquired Pneumonia	As above, but supported by chest radiograph findings of lung shadowing that is likely to be new. In the elderly, the presence of chest radiograph shadowing accompanied by acute clinical illness without other obvious cause.
Scott et al, 2000 (6)	*Community Acquired Pneumonia*	An illness of 14 days duration or less that consisted of at least two respiratory symptoms (cough, sputum, breathlessness, chest pain, haemoptysis, or fever) with evidence of pulmonary consolidation on chest radiograph.
Farr et al, 2000 (209)	*Community Acquired Pneumonia*	Acute respiratory illness with radiological pulmonary shadowing which was at least segmental or present in more than one lobe.
Almirall et al, 2008 (216)	*Community Acquired Pneumonia*	Acute lower respiratory tract infection for which antibiotics had been prescribed, in association with the appearance of previously unrecorded focal signs on physical examination of the chest and new radiological findings suggestive of pneumonia infiltrate.

2.3.1.4. Management and prognosis

Management varies according to severity and aetiology, with the mainstay of treatment for bacterial pneumonia being antibiotics (oral or intravenous, depending on severity), escalating to supplementary oxygen, circulatory support, and ventilatory support in a high dependency or intensive care unit as required.

Severity assessment can be made using a variety of clinical scores – such as CURB65, CURB, SMRT-CO and Pneumonia Severity Index – which have been validated for use in different populations in well resourced-settings (217). Such tools may not be valid in low-income settings such as sub-Saharan Africa, where the patient demographics and underlying aetiologies are likely to be different. CURB65, SMRT-CO and the IDSA/ATS minor criteria – which were all derived from community acquired pneumonia populations in well-resourced settings – performed poorly as predictors for mortality in the MARISO (Malawian Adult Lower Respiratory Tract Infection Severity, Aetiology and Outcome Study) cohort (218). Reasons for this may include the different age of the pneumonia cohort, the impact of advanced immunosuppression and malnutrition, as well as altered healthcare seeking behaviours and under-resourced healthcare facilities leading to delays in treatment. The MOST score – derived from the MARISO cohort – uses simple parameters (male sex, oxygen saturation, inability to stand and tachycardia) and provides good discriminative capability for the prediction of 30-day mortality (area under the receiver-operating curve 0.79, 95% CI 0.73-0.85) but requires external validation in another sub-Saharan Africa setting.

A meta-analysis of 127 unique study cohorts found an overall mortality rate for community-acquired pneumonia of 13.7%, ranging from 5.1% in ambulatory and hospitalised patients to 36.5% in intensive care patients (219). The mortality rate varied widely according to aetiology. More recently, a study in Spain found a mortality rate of 17.0% (95% CI 16.9-17.1%) for all-cause community-acquired pneumonia in hospital (220), suggestion little improvement in mortality despite advances in treatment in recent decades. In Europe, mortality has been found to be associated with advanced age, co-morbid conditions, female gender, microbial aetiology, multi-lobar involvement and community acquired pneumonia severity (205). Mortality risk in elderly patients persists for several years following an episode of pneumonia, even when pre-existing comorbidities are accounted for (221).

2.3.2. Risk factors for pneumonia

The risk factors for developing pneumonia in developed countries are well described, with old age, male sex, comorbidities (including chronic respiratory diseases, immunosuppression, congestive heart failure, cerebrovascular disease and diabetes) and lifestyle factors such as tobacco smoking

and high alcohol intake being frequently being cited (208, 209, 222-224). Being underweight or having poor dental hygiene have also been identified as risk factors for pneumonia, as has contact with young children (216, 225, 226). A large case-control study of community-acquired pneumonia in Spain found a wide range of risk factors to be statistically significant in multivariate analysis, including epilepsy (OR 5.95, CI 1.62-21.74), sudden temperature changes at work (OR 2.64, CI 1.67 – 4.15), oxygen therapy within the last year (OR 2.42, CI 1.16-5.05), upper respiratory infections in the last month (OR 2.28, CI 1.81-2.89), chronic bronchitis (OR 1.81, CI 1.19-2.75), asthma (OR 1.67, CI 1.28-2.19), contact with young children (OR 1.48, CI 1.2-1.82), and smoking (>150 pack years: OR 1.46, 95% CI 1.14-1.86) amongst others (216).

2.3.2.1. HIV

HIV is associated with an up to 25-fold increase in bacterial pneumonia rates (23-25) and a reduction in CD4 cells increases the risk of pneumonia (26, 227). In a cohort study of female sex-workers (81.6% of women were HIV seropositive) in Kenya in the pre-antiretroviral era, the relative risk of developing acute pneumonia with HIV infection was 17.1 (95% CI 2.4-121.8) (24). In the HIV-positive women, 35.5% of these pneumonias were confirmed to be pneumococcal. Women with a first presentation of pneumonia had a mean CD4 count of 329cells/µl compared to 204 cells/µl in recurrent episodes of pneumonia. Antiretroviral therapy does not provide full pneumococcal protection (228): the risk of pneumonia in HIV infected individuals remains elevated even when on antiretroviral therapy (229), and individuals with HIV commonly present with pneumonia even when their CD4 count is in the normal range (26). Unsurprisingly, therefore, despite reductions in pneumonia since the roll-out of antiretroviral therapy in some parts of sub-Saharan Africa (230), pneumonia remains one of the commonest reasons for admission to hospital in HIV endemic areas (5).

Community-acquired pneumonia is commonly complicated by other HIV-associated infections, in particular tuberculosis (231), raising challenges for empirical treatment regimens in high HIV-prevalence settings (29). There is a lack of available data regarding whether this major risk factor for pneumonia interacts with the effects of household air pollution.

2.3.2.2. Tobacco smoke exposure

Risk of pneumonia is increased in individuals who have ever smoked (OR 2.0, 95% CI 1.24-3.24); for current smokers and ex-smokers the ORs were 1.88 (95% CI 1.11-3.19) and 2.14 (95% CI 1.26-3.65), respectively (14). The same study, conducted in the 1990's in Spain, found that the proportion of community acquired pneumonia attributable to smoking in all cases was 32.4% (95% CI 14.8-50.1%), reduced to 23% (95% CI 3.3-42.7%) in cases who did not have a co-existing history of COPD. A dose-

response relationship between smoking pack-years and community acquired pneumonia was also identified. Although community acquired pneumonia risk was increased in ex-smokers, this risk reduced after 5 years of cessation (1-4 years cessation OR 3.52, 95% CI 1.72-7.17; 5-9 years cessation OR 1.21, 95% 0.48-3.04) suggesting a possible reversal of airway damage, although only small numbers of participants were included in these sub-groups.

Tobacco smoke is known to cause histological changes in the airway epithelium creating conditions more favourable for microbial survival (232-235), and induces an inflammatory reaction triggering innate and adaptive immune responses (236, 237). Key immune signalling pathways, including the response of Toll-like receptor 2 and nuclear factor kappaB are inhibited (238, 239), as are opsonisation and phagocytic functions (240): this modulation of the lung's immune response to pathological organisms increases susceptibility to infection.

2.3.3. Pneumonia in African adults

There are estimated to be 4 million episodes of pneumonia in adults in Africa causing 200,000 deaths per year (19). Pneumonia has been one of the commonest causes of adult admission to hospital in both the pre- and post-antiretroviral era (5, 20). Unlike in developed settings, young adults are affected (over half of patients were 35 years or younger in two large African cohorts), with an increased prevalence in males (6, 218); the effect of pneumonia on the working-age population results in a large economic burden (19).

2.3.3.1. Aetiology and prognosis of pneumonia in African adults

Few studies detailing the aetiology and outcomes of pneumonia in Africa have been published. The most comprehensive study published to date, by Scott *et al,* evaluated 281 adults with radiologically confirmed pneumonia in urban and rural Kenya between 1994-1996 (6). The majority of patients were male (63%) and 52% were HIV seropositive; patients presenting to an urban hospital were more likely to be male and HIV-positive than those presenting to a rural hospital. Lung aspiration was performed on 259 patients, blood cultures were performed on all and 275 had urinary pneumococcal antigen testing: 146 patients (52%) had a positive lung aspirate or blood culture or pneumococcal antigen – of those, 6 patients had dual culture positivity. 37 patients (13%) were found to be Mycobacterial culture or polymerase chain reaction (PCR) positive (14 of whom also had blood or lung aspirate culture positivity) and 23 patients (8%) had positive viral or atypical serology (10 of whom also had blood or lung aspirate culture positivity). Two patients had a positive lung aspirate or blood culture, positive Mycobacterial culture or PCR and positive viral or atypical serology. An aetiological agent was identified in 65% of patients: *Streptococcus pneumoniae* infection was the most commonly identified pathogen, found in 46 % of patients. Other pathogens

identified included *Staphylococcus aureus* (1.4%), *Haemophilus influenzae* (3.6%), *Salmonella* species (2.1%), other Gram-negative bacteria (1.4%), *Mycobacterium tuberculosis* (8.9%), Non-tuberculous mycobacteria (3.6%), *Nocardia* species (0.4%), *Mycoplasma pneumoniae* (2.5%), Influenza A or B virus (5%), and Adenovirus (0.7%). *Mycobacterium tuberculosis* was detected almost twice as frequently from HIV-positive patients than HIV-negative patients. Of 255 patients in whom the outcome at 3 weeks was known, 176 (69%) recovered, 34 (13%) were on anti-tuberculous therapy, 20 (8%) complained of ongoing respiratory symptoms and 25 (10%) had died. Factors associated with death within 3 weeks of acute pneumonia in a multivariate analysis included age (OR 1.51, 95% CI 1.04-2.19), unemployment (OR 4.42, 95% CI 1.21-16.1), prior visit to a traditional healer (OR 5.26, 95% CI 1.67-16.5), prior visit to a pharmacy (OR 0.30, 95% CI 0.10-0.91), Herpes labialis (OR 15.4, 95% CI 2.22-107) and heart rate (per 10 beats/min rise) (OR 1.64, 95% 1.24-2.16).

In a study of pneumococcal pneumonia in Malawi in the pre-antiretroviral era, the presence of multi-lobar chest signs was associated with a worse prognosis at follow-up compared to those with unilateral signs (OR 6.6, 95% CI 2.3-19.0) (241). There was 20% inpatient mortality, and hypotension at presentation was associated with in patient death (OR 10.4, 95% CI 1.8-60.6).

In the MARISO study, a recent prospective cohort study of 459 hospitalised pneumonia patients in Blantyre, Malawi, there was a male preponderance (62%), median age was 34.6 years and HIV-prevalence was 78% (218). In patients with a symptom duration of 14 days or less, *Mycobacterium tuberculosis* and *Streptococcus pneumoniae* were the most commonly identified pathogens (23% and 21%, respectively). This high prevalence of tuberculosis amongst individuals presenting with acute pneumonia is a striking finding, which adds to the growing body of evidence which challenges the conventional understanding that tuberculosis presents with insidious symptoms (6, 242). Mortality at 30 days was 14.6%, with increased mortality associated with male sex (adjusted Odds Ratio (aOR) 2.57), increased length of pre-presentation symptom duration (aOR 1.11 per day increase), inability to stand (aOR 4.38), heart rate (aOR1.02 per beat/minute rise), oxygen saturations (aOR 0.95 per % rise), white cell count (aOR 0.91 per 10^9/L rise) and haemoglobin (aOR 0.90 per g/dL rise).

A systematic review of pneumonia studies from sub-Saharan Africa identified 44 studies for inclusion, with 39 studies providing mortality data and 30 studies providing aetiological data (218). Pooled data found an average age of 38 years and HIV prevalence of 52%. *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* were identified in 27% and 19% of cases respectively. The overall mortality rate in hospitalized patients was 9.5%.

2.3.3.2. Risk factors for pneumonia in African adults

Risk factors for developing pneumonia are less well understood in developing countries like Malawi where HIV has dominated the recent picture. As HIV management improves and living with HIV becomes life with a chronic disease, risk factors for pneumonia other than HIV will become increasingly important. Poverty and crowded living conditions have previously been identified as risk factors for adult pneumonia in sub-Saharan Africa (227, 243). Many other preventable exposures may play a role but are unproven in this setting, including air pollution exposure, chronic respiratory disease (CRD), tobacco smoke, malnutrition, excess alcohol and animal ownership (12, 14, 209, 216, 244-250).

Household air pollution is an established risk factor for childhood ALRI, increasing a child's risk of ALRI by 78% (pooled OR = 1.78 (95% CI 1.45 to 2.18) (12). Half a million deaths per year from childhood pneumonia are thought to be caused by exposure to household air pollution (12). As discussed in Chapter 3, the risk in adults is yet to be established, and the impact of HIV infection on that risk is unknown. As the burden of pneumonia and air pollution is high in sub-Saharan Africa, if an association present the attributable risk is potentially very high.

2.3.4. Conclusion

Pneumonia causes a high burden of morbidity and mortality in sub-Saharan Africa, but the majority of the literature describing aetiology, risk factors and prognosis for pneumonia is from well-resourced settings. The picture of pneumonia in sub-Saharan Africa is different, due to differences in demographics, social factors, comorbidities and health care provision. Several potential risk factors, including household air pollution, are modifiable and therefore provide potential opportunities for targeted reduction of pneumonia risk. However, before resources are directed towards specific interventions, the risk factor profile in sub-Saharan Africa should be accurately described.

3. Systematic Review – Is household air pollution a risk factor for Acute Lower Respiratory Tract Infections in adults?

3.1. Introduction

As discussed in Chapter 2, disease outcomes in adults attributed to household air pollution from solid fuels include ALRI in children, COPD and cardiovascular disease (7). Based on the evidence for an association between household air pollution and child ALRI (12), an association between household air pollution and adult ALRI is plausible, but this lacks strong evidence. This is an important gap in the evidence base: although the Global Burden of Disease study incorporated adult ALRI as an outcome for household air pollution exposure, the risk estimate was derived from an integrated exposure-response curve for $PM_{2.5}$ from ambient and household air pollution, and tobacco smoke (7, 9, 11). This extrapolation simplifies and risks misclassifying the exposure-response relationship, because it ignores the fact that health risks may vary depending on the combustion source, due to the heterogeneity of constituents found in $PM_{2.5}$ (66). If an association is confirmed then the attributable risk is potentially high, because household air pollution exposure and ALRI burden are both concentrated in developing countries. This chapter follows on from the narrative literature reviews presented in Chapter 2 and reports on a systematic review of the literature on household air pollution and ALRI in adults.

3.1.1. Objectives

To describe and summarise the evidence base for the relationship between household air pollution exposure from domestic solid fuel use and adult ALRI worldwide, to identify knowledge gaps and future research opportunities.

3.1.2. Contributors to this chapter

I developed the protocol for this systematic review with input from Dr Deborah Havens (Liverpool School of Tropical Medicine, (LSTM), UK), Dr Daniel Pope (University of Liverpool, UK) and Professor Nigel Bruce (University of Liverpool, UK). I performed all database and grey literature searching and collated the full list of titles. Title searching was performed by myself, Deborah Havens and Hope Simpson (London School of Hygiene and Tropical Medicine, UK). I reviewed all abstracts and full text articles and the second review was performed by either Hope Simpson, Deborah Havens or Dr Geoffrey Manda (University of Malawi, Malawi). I extracted data, with assistance from Hope Simpson, and I collated the data and performed all quality assessments and risk of bias assessments. I wrote the manuscript for publication, with assistance from Hope Simpson, and editing input from

Deborah Havens, Daniel Pope, Nigel Bruce and Dr Kevin Mortimer (LSTM, UK). Translation of French and Spanish language manuscripts was performed by Dr Christine Kelly (University of Liverpool, UK) and Mariana Gallo, respectively.

3.2. Methods

This systematic review is registered with the Centre for Reviews and Dissemination (Registration number: CRD42015028042). The full protocol is available at http://www.crd.york.ac.uk/prospero.

3.2.1. Eligibility criteria

The participants, exposures and outcomes of interest are defined below. All studies were included in the review if they provided an effect estimate for the exposure of interest on the outcome for relevant participants, in the form of a relative risk or OR with 95% CI (or data allowing calculation), and met the following criteria:

- Human studies.
- Study designs: Individually- and cluster-randomised control trials; controlled before-and-after trials; cohort studies; case-control studies and cross-sectional surveys were included. Case reports, case series and review articles were excluded.
- English language papers or non-English language papers where a translation is available (translation was not available for Chinese literature).

3.2.1.1. Participants

All adults (age ≥18 years old) were eligible, without geographical restriction. Studies exclusively focussed on children (<18 years) were excluded. Studies including both adults and children were included if the data was presented separately. To avoid the exclusion of potentially useful data on adults, studies that included low numbers of under 18-year olds within a primarily adult population were included.

3.2.1.2. Exposure

Exposure was defined as air pollution from indoor burning of any solid fuels - including wood, charcoal, animal dung, crop residues and coal - for household purposes. We included studies that quantified exposure through direct measurement of specific pollutants, questionnaires regarding exposure history, comparison of groups exposed to types of exposure (*e.g*. different stove types), or before and after an intervention to reduce exposure. Studies examining outdoor air pollution, occupational exposures, non-fuel combustion sources or non-solid fuels were excluded. Non-fuel combustion sources in households, such as insect coils, air fresheners or tobacco smoke, were also excluded as the exposures, health effects and control measures for these pollution sources are likely

to differ significantly from that of solid fuel (58). Exposure assessments or interventions may be implemented at an individual or household level.

3.2.1.3. Outcomes

The primary outcome of interest was ALRI including pneumonia, acute bronchitis or bronchiolitis, assessed by any of the following:

- Physician diagnosed ALRI
- Patient/caregiver recall of ALRI diagnosed by a physician
- Patient/caregiver recall of key symptoms (recall of up to 14 days) and direct observation of signs compatible with ALRI by a health worker / staff trained by WHO guidelines, as follows:
    - Symptoms: cough plus one of dyspnoea, pleuritic pain
    - New focal chest signs on examination (*e.g*. Bronchial breathing)
    - Systemic Signs: one of sweating, fever, shivers, myalgia or pyrexia >38°C
    - No other explanation for the illness
- Patient/caregiver recall of key symptoms and signs (recall of up to 14 days)
- Physician issued death certificate indicating ALRI
- Verbal autopsy diagnosis of ALRI

Chest X-ray findings and positive blood/sputum culture results improve diagnostic certainty and so were considered to provide a higher quality of evidence, but were not essential for the purpose of this review.

Studies that defined the outcome as “acute” or specified duration of less than 14 days were included, even if infection was not confirmed, since acute respiratory illnesses in the absence of underlying disease is usually infectious in origin. Studies reporting illnesses lasting longer than 14 days were excluded, to avoid inclusion of chronic diseases or chronic infections such as tuberculosis. Studies examining exacerbations of chronic conditions such as asthma or COPD were included if an acute infectious exacerbation was defined. Studies that did not distinguish between upper respiratory tract infections and ALRI were excluded.

3.2.2. Search strategy

The databases listed in Box 3-1 were searched on 17/12/2015 using the search terms and Boolean phrases listed in Box 3-2. An example search strategy is shown in Box 3-3. Searches were limited to papers published from 01/01/1960 onwards, and to human studies where possible. Identified papers were imported into EndNote X7.

Box 3-1. Databases searched

- MEDLINE (OVID)
- Scopus (including EMBASE)
- Web of Science
- CINAHL (Ovid)
- Global Health (Ovid)
- Cochrane Central Register of Controlled Trials (CENTRAL)
- Database of Abstracts of Reviews of Effects (DARE)
- Latin American and Caribbean literature (LILACS)
- SciELO
- African Index Medicus

Alternative sources were also searched for eligible studies:

- www.who.int/trialsearch
- www.clinicaltrials.gov
- EAGLE (European Association for Grey Literature Exploitation), www.opengrey.eu
- Reference lists of all selected papers were reviewed for any potentially eligible titles.
- Reference lists of relevant review articles were reviewed for any potentially eligible titles.

Following removal of duplicates, titles of identified papers were reviewed for eligibility. The first 20% of titles were reviewed in duplicate by two independent authors to check for agreement, and the remaining titles were reviewed by a single author, with an overlap of 10% of titles between authors to ensure continued agreement.

Abstracts of the selected titles and then full text of the selected articles were reviewed for eligibility, according to the selection criteria. Abstracts and full text articles were all reviewed in duplicate by two independent authors. Where discordant decisions were not resolved by discussion, the decision was made by a third author.

If the full text article was not available in English, translation was sought to determine eligibility.

For studies where relevant details were not available, authors were invited to supply information if their contact details were available.

Box 3-2. Search terms and Boolean phrases used

Outcomes (**OR**): "respiratory infection*", "respiratory tract infection*", pneumonia, "respiratory illness*"

AND

Exposures/Interventions (**OR**): "household air", "indoor air", biomass,*smoke, fuel*, *stove*.

Box 3-3. Example search strategy for Medline (OVID)

1. "respiratory infection*".mp,tw.
2. "respiratory tract infection*".mp,tw.
3. pneumonia.tw,mp.
4. "respiratory illness".tw,mp.
5. 1 or 2 or 3 or 4
6. "household air".tw,mp.
7. "indoor air".tw,mp.
8. biomass.tw,mp.
9. smoke,tw,mp.
10. stove.tw,mp.
11. fuel.tw,mp.
12. 6 or 7 or 8 or 9 or 10 or 11
13. 5 and 12

3.2.3. Data extraction

Data were extracted from the selected papers using a previously piloted data extraction form, from which a summary table and narrative synthesis were produced. Where effect estimates were not provided, these were calculated from the published data.

3.2.4. Quality assessment

Studies that quantified exposure by direct household or personal measurement of specific pollutants were regarded to provide a higher quality of exposure assessment than those that relied on self-reported exposure.

Studies using prospective assessment of ALRI by a physician or trained health care worker using predefined criteria or diagnostic investigations were considered to be of higher quality than those reliant of self-reported episodes of ALRI.

3.2.5. Risk of bias assessment

Risk of bias was assessed using the Liverpool Quality Assessment Tool (see Section 9. Appendix 1), which reviewed the study methodology in four domains - subject selection, exposure assessment, outcome assessment and adjustment for confounders - and assigned quality ratings (low, moderate or high risk of bias). I adopted this instead of the Cochrane Collaboration's tool for assessing risk of bias due to the variety of study designs identified. Publication bias was not assessed due to the small number of papers identified.

3.2.6. Data analysis

Methodological heterogeneity in exposure and outcome assessment precluded a meta-analysis, but a narrative summary of the selected papers and a summary of findings table were produced.

3.3. Results

3.3.1. Summary of literature search

Database searches identified 8605 records and 330 records were identified from alternative sources. After removal of duplicates 4616 records remained. The number of titles, abstracts and papers reviewed was 4616, 512 and 72 respectively, as summarised in the PRISMA flow chart (Figure 3-1), with the reasons for exclusion at the abstract and full text stages. Agreement between the 3 independent authors was 95-96% for the first 20% of titles reviewed, and 95-98% for the 10% overlap of remaining titles. It was not possible to locate 5 selected abstracts (251-255) and 2 selected full text papers - both published in Chinese (256, 257) - for review. Two authors were contacted for further details about their recent or ongoing studies identified at www.clinicaltrials.gov. One study is a prospective cohort study of febrile adults in Tanzania for which recruitment has been completed but the data are not yet available. The second study is a randomized trial of smoke reduction interventions in Native American populations, but we were

unable to obtain further details. One further study identified, the case-control study presented in Chapter 6 (the AIR study), was not included at results were not yet available.

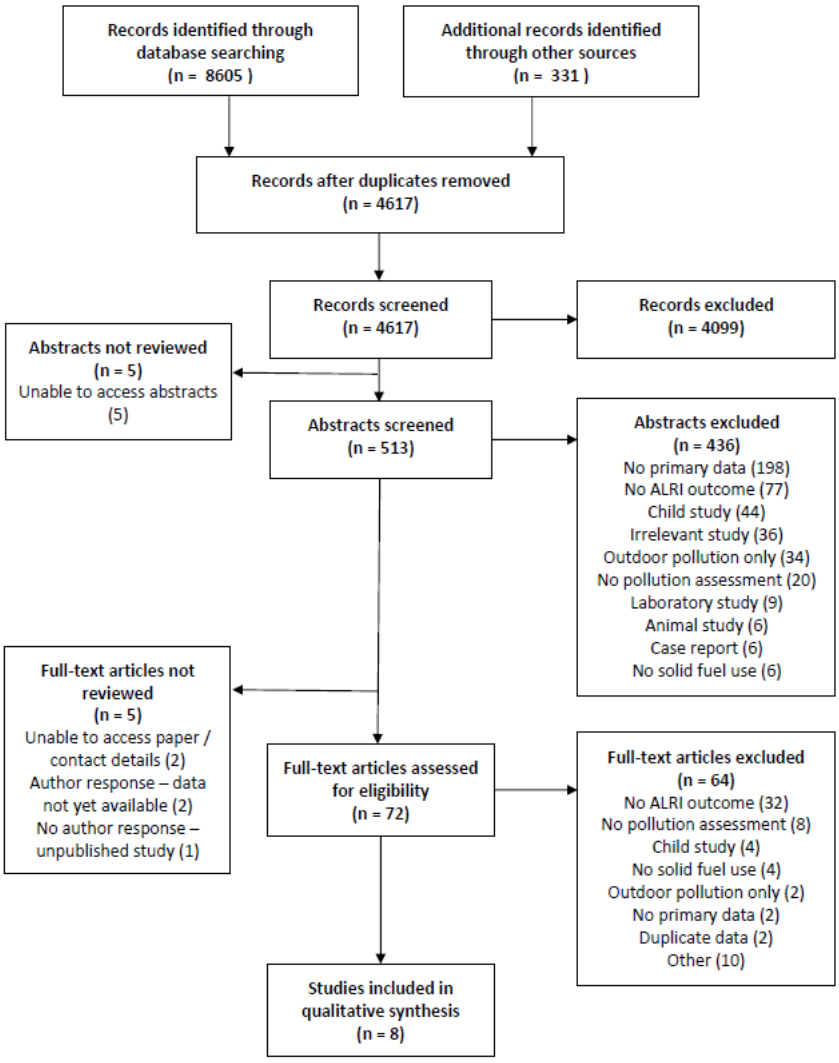

Figure 3-1: Systematic review PRISMA flow chart.
ALRI: acute lower respiratory tract infection

Ten papers met the criteria for inclusion; data from 2 of these were duplicated in other selected papers (258, 259), and therefore not extracted.

The main findings of all included studies are summarised in Table 3-1, which demonstrates the heterogeneity of study participants, exposure definitions and outcome definitions.

3.3.2. Study designs

The 8 selected papers included 4 cross-sectional (260-263), 2 cohort (264, 265) and 2 case control studies (266, 267). The largest study was a retrospective cohort study from China, which reported on the causes of death in 42,422 farmers (264).

3.3.3. Study settings

The articles covered study locations from 8 countries (Kenya, Tanzania, Sierra Leone, China, Nepal, Canada, Mexico and Serbia) across 4 continents. Most studies were based in rural settings and recruited participants from communities. There were 3 exceptions, which were hospital or health-centre based studies, conducted in urban areas of Canada, Mexico and Serbia (262, 266, 267).

3.3.4. Study participants

Most studies predominantly or exclusively recruited females (accounting for 48-100% of participants within studies). In the study from Tanzania, the cooks were mainly female but the gender and age of the unexposed group were not reported (261).

Except for the study from Canada, which restricted recruitment to individuals over 65 (266), and the study from China (264), in which deaths occurred between 25 and 80 years of age, all studies recruited younger adults. Five studies restricted participants to adults over 18, whilst the 3 remaining studies - from Sierra Leone, Tanzania and Kenya - included younger participants but were conducted in predominantly adult populations (261, 263, 265).

3.3.5. Exposure assessments

One study evaluated coal use (Shen *et al*) and the 7 others evaluated biomass fuel use, predominantly wood. Four studies measured levels of household pollutants directly, but only 1 presented an estimate for the effect of pollutants on ALRI risk. In Kenya, Ezzati *et al.* measured particulate matter <10µm diameter (PM_{10}) for 14-15 hours per day for 200 days in 55 households, and demonstrated a dose-response relationship between exposure to PM_{10} and ALRI risk (265). All studies monitoring air pollution levels measured PM_{10} rather than $PM_{2.5}$. This may misrepresent the true harmful exposure, as smaller $PM_{2.5}$ particles are inhaled deeper in to the lungs than PM_{10} and are therefore thought to be more pathogenic (43).

The 7 studies that did not provide an effect estimate for measured pollutants all used questionnaire data to classify exposure to household air pollution, based on a variety of self-reported measures including fuel or stove type, quantification of fuel use, ventilation, cooking frequency and location.

Table 3-1: Summary of findings table for four studies investigating the effects of household air pollution on adult acute lower respiratory tract infections.

Study	Sample selection	Sample size	Subject description	Exposure	Outcome	Adjustment for confounders	Effect size
COHORT STUDIES							
Ezzati, 2001 (265) **Kenya, rural** **Conducted 1996-1999**	55 randomly chosen households Response rates of household members not stated	229 individuals	47.6% 5-14 years 52.4% 15-49 years (Age groups treated as one category, but ALRI more common in the latter) 55% female	Continuous measurement of PM_{10} in households for 14-15 hours a day for 200 days	ALRI (including bronchitis, pneumonia and broncho-pneumonia) diagnosed by a nurse who visited all households every 1-2 weeks for 2 years, and examined anybody reported to have respiratory symptoms	Adjusted for age, sex, smoking, village, and household occupancy. No adjustment for socioeconomic status, but homogenous group of participants	Adjusted logistic regression, OR (95% CI, p value): Reference category: <200µg/m^3 PM_{10} 200-500 µg/m^3: 1.65 (0.5-5.45, 0.41) 500-1000 µg/m^3 1.87 (0.6- 5.71, 0.27) 1000-2000µg/m^3 2.74 {0.93-8.12, 0.07) 2000-4000µg/m^3 3.28 (1.09-9.85, 0.03) 4000-7000 µg/m^3: 3.21 (1.01-10.24, 0.05) >7000 µg/m3: 7.10 (2.26-22.32, 0.001)
Shen, 2009 (264) **China, rural** **Conducted 1992-1996**	Identified from local administrative records	44,850 individuals identified 42,422 followed up to endpoint	All famers in 4 communes born between 1917 and 1951 and living in Xuanwei on 1/1/1976 49% female	Principal use of smoky or smokeless coal, and presence of chimney, assessed by standardised questionnaire	Death from pneumonia obtained from public records of death certificates, which were completed by physicians	Adjusted for annual coal use, stove improvement, smoking, years of cooking, education, house size and occupancy, coal mining, COPD and time spent indoors	Cox proportional hazards model, HR (95%CI, p value): Stove improvement vs no stove improvement Men: 0.49 (0.31-0.78, 0.002) Women: 0.53 (0.32-0.88, 0.014) Smokeless coal vs smoky coal Men: 1.52 (0.98-2.36, 0.060) Women: 1.44 (0.90-2.31, 0.129)

Continued overleaf

Table 3-1 continued Study	Sample selection	Sample size	Subject description	Exposure	Outcome	Adjustment for confounders	Effect size
CASE CONTROL STUDIES							
Loeb, 2009 (266) **Canada, urban** **Conducted 2002-2005**	Cases were patients who presented to emergency departments. Not stated if all consecutive patients recruited. Controls recruited contemporaneously using random-digit dialling. Response rates not reported	717 cases 867 controls	Cases: pneumonia patients, 40% female, age >65 (mean 79) Controls: unmatched healthy community controls, 68% female, age >65 (mean 74) The same geographical restrictions were used for both groups	Recall of fireplace use in previous 12 months, assessed by structured questionnaire. No quantification of exposure	Pneumonia, based on appropriate clinical criteria plus radiographic findings, all performed by physicians	Backward-stepwise logistic regression performed adjusting for multiple variables but fireplace use not retained in the final model so only unadjusted results reported	Unadjusted logistic regression, OR (95%CI, p value): Fireplace use vs. no fireplace use 0.69 (0.54-0.87, 0.002)
Figueroa, 2012 (267) **Mexico, urban** **Conducted 2000-2007**	Retrospective review of hospital records and social work department records. Implied that all consecutive records included but not implicitly stated	948 cases 1305 controls	Cases: bacterial pneumonia, 42% female, age >18 (mean 55) Controls: otolaryngeal patients, 38% female, age >18 (mean 32)	Past or current exposure to woodsmoke in the home, based on retrospective review of secondary source documents. No quantification of exposure	Bacterial pneumonia, based on retrospective review of hospital records by specialists using a standardised format and clinical definition of pneumonia	Adjusted for age, gender, occupational exposures, Type 2 Diabetes, smoking, and household ventilation. No adjustment for socioeconomic status or outdoor exposures	Logistic regression, OR (95% CI, p value): (unadjusted results calculated from raw data) Current wood smoke exposure vs No current wood smoke Unadjusted: 2.62 (1.78-3.86, <0.0001) Past wood smoke exposure vs No past wood smoke Unadjusted: 2.14 (1.89-2.55, <0.0001) Adjusted: 1.1 (0.9-1.4, 0.5)

Continued overleaf

Table 3-1 continued Study	Sample selection	Sample size	Subject description	Exposure	Outcome	Adjustment for confounders	Effect size
CROSS-SECTIONAL STUDIES							
Shrestha, 2005 (260) **Nepal, rural (81%) & urban (19%)** **Conducted 2003-2004**	Households randomly selected Household member response rates not stated	98 households 168 respondents	94% female, mean age 36 years (S.D. 16.7), minimum age not stated	Use of "unprocessed fuel" (solid bio-fuels) vs "processed fuels" (gas / kerosene), assessed by questionnaire	ALRI, based on physician examination and review of symptoms but no definition stated. Not stated whether retrospective diagnoses included	No adjustment for confounders	Unadjusted OR (95% CI): Unprocessed fuel vs processed fuel use 2.69 (0.76-9.52)
Kilabuko, (2007) (261) **Tanzania, rural** **Conducted 2004**	Random sampling of household No refusal rates for household members stated	100 households 390 participants	No demographics of participants stated, but includes age 5 and over "Chief cooks" were mainly wives of household heads, so assumed predominantly adult but not necessarily	"Chief cooks" compared to other household members. No explanation of how chief cooks were chosen or defined. Likely significant overlaps between 2 groups. No quantification of exposure	ARI, defined as household member reported cough with rapid breathing, assessed by questionnaire. No recall period defined	No adjustment for confounders	Unadjusted OR (95% CI, p value) calculated from raw data Chief cook vs other household members 3.76 (2.19-6.48, <0.0001)

Continued overleaf

Table 3-1 continued Study	Sample selection	Sample size	Subject description	Exposure	Outcome	Adjustment for confounders	Effect size
CROSS-SECTIONAL STUDIES continued							
Stanković, 2011 (262) **Serbia, urban.** **Conducted 2008.**	Individuals recruited from a health centre when attending for health checks. No description of how the sample was selected. Response rates not reported	1082 participants	All female, age 20-40 Smokers and occupational exposures excluded	Self-reported 'use of biomass fuels', assessed by questionnaire. No quantification of exposure	Self-reported "doctor diagnosed pneumonia' or 'doctor diagnosed bronchitis' in their life time, assessed by questionnaire	Adjusted for age, education, family history of respiratory illness and outdoor air pollution. Not explicit whether adjusted for environmental tobacco smoke, home dampness or pets. Not adjusted for comorbidities or socioeconomic factors	Adjusted logistic regression, OR (95% CI) Biomass use vs no biomass use Bronchitis: 0.91 (0.71 - 1.15). Pneumonia: 0.99 (0.80 - 1.22).
Taylor, 2012 (263) **Sierra Leone, rural and peri-urban** **Conducted 2011**	Participants randomly selected from all eligible individuals in the study area in 16 community strata, using stratified sampling. Response rates not stated	520 participants	All female, age 15-45	Kitchen location, type of fuel normally used and number of hours spent in the kitchen, assessed by questionnaire	ARI, defined as self-reported cough followed by rapid breathing, assessed by questionnaire with a 2 week recall period Not adjusted for other pollution exposures comorbidities or socio-economic factors	Adjusted for age, marital status, kitchen type, smoking, housing type and number of rooms.	Logistic regression, OR (95% CI, p value): Wood vs charcoal Adjusted: 1.14 (0.71 - 1.82, 0.580) Kitchen inside the main house vs separate kitchen Adjusted: 0.68 (0.38 - 1.24, 0.210) Effect of spending 4-6 hours in kitchen or >7 hours vs <3 hours Unadjusted: 1.31 (0.88-1.94, 0.179) and 2.40 (0.86- 6.72, 0.094), respectively.

PM_{10} = Particulate Matter < 10µm diameter; ALRI = Acute Lower Respiratory Infection; ARI = Acute Respiratory Infection; COPD = Chronic Obstructive Pulmonary Disease; OR = Odds Ratio; HR = Hazard Ratio; CI = Confidence Interval

3.3.6. Outcome assessments

Two studies defined the outcome using prospective health care worker diagnosis of ALRI; in Canada, hospitalised cases had symptoms or signs, and radiological changes consistent with pneumonia (266); in Kenya, the outcome was defined as nurse-diagnosed ALRI (including bronchitis, pneumonia or broncho-pneumonia) following review and physical examination at 1-2 weekly visits over a 2 year period (265). A third study, from Nepal, included a physician's review to diagnose ALRI, but no further definition was provided and it is unclear whether retrospective diagnoses were included (260). The study from China used retrospective review of death certificate records, which had been completed by physicians on the basis of clinical and radiological investigations (264). Similarly, the study in Mexico used a retrospective review of hospital records by specialists to identify cases (267). The remaining studies used self-reported symptoms or past diagnoses, with varying recall periods, to define the outcome (261-263).

3.3.7. Adjustment for confounders

Five of the 8 studies made some adjustment for potential confounders in their analysis of the effect of household air pollution on ALRI (262-265, 267), but several studies failed to make adjustments for important potential confounders, such as economic status and smoking.

3.3.8. Effect estimates

The effect estimates quantified by the studies are shown in Table 3-1. There were inconsistent findings across the studies. Whilst the 2 cohort studies have marked differences in study design, they demonstrated statistically significant harmful effects of household air pollution exposure on ALRI after adjustment for confounders. A dose-dependent relationship between PM_{10} and ALRI was detected in Kenya, (OR=7.10, (95% CI 2.26 to 22.32) for exposure to >7000µg/m^3 PM_{10} (highest exposure category) vs. <200 µg/m^3 PM_{10}), and a reduction in risk of pneumonia deaths was observed in participants using an improved coal stove instead of a traditional coal stove (OR=0.49 (95% CI 0.31 to 0.80) and 0.53 (95% CI 0.32 to 0.88)) in Chinese men and women respectively (264). The studies from Mexico and Tanzania both reported harmful effects of household air pollution exposure in univariate analysis (261, 267), but these were no longer significant after adjustment for confounders in the Mexican study. The studies from Nepal, Serbia and Sierra Leone did not identify a significant association between household air pollution and risk of ALRI (260, 262, 263). The study from Canada found that fireplace use (no further details provided) was associated with a reduction in the risk of pneumonia, although this was unadjusted (266).

3.3.9. Quality and Risk of Bias assessment

The methodological quality of each study is summarised in Table 3-2, according to 4 domains of study design. An overall risk of bias is given, based on assessment of the sources of potential study bias found. The 2 cohort studies were considered to be of a generally higher standard, with a lower risk of bias, than the other studies, which were all thought to have a high risk of bias in at least 2 of 4 domains.

Table 3-2: Assessment of risk of bias, by four domains of methodology, using the Liverpool Quality Assessment Tool.

Study	Subject Selection	Exposure Assessment	Outcome Assessment	Adjustment for Confounding	Overall Risk of Bias
COHORT STUDIES					
Ezzati, 2001 **Kenya, rural**	MODERATE	LOW	MODERATE	MODERATE	MODERATE
Shen, 2009 **China, rural**	LOW	MODERATE	MODERATE	LOW	LOW / MODERATE
CASE CONTROL STUDIES					
Loeb, 2009 **Canada, urban**	MODERATE	HIGH	LOW	HIGH	MODERATE / HIGH
Figueroa, 2012 **Mexico, urban**	HIGH	HIGH	MODERATE	MODERATE	MODERATE / HIGH
CROSS-SECTIONAL STUDIES					
Shrestha, 2005 **Nepal, rural (81%) & urban (19%)**	MODERATE	HIGH	MODERATE	HIGH	MODERATE / HIGH
Kilabuko, (2007) **Tanzania, rural**	MODERATE	HIGH	HIGH	HIGH	HIGH
Stanković, 2011 **Serbia, urban.**	HIGH	HIGH	HIGH	MODERATE	HIGH
Taylor, 2012 **Sierra Leone, rural and peri-urban**	MODERATE	HIGH	HIGH	MODERATE	MODERATE / HIGH

The two studies with the lowest overall risk of bias (low/moderate or moderate rating) both found a positive association between air pollution exposure and ALRI risk in adjusted analyses (264, 265). Studies with an overall moderate/high risk of bias found either a negative association between air pollution exposure and ALRI risk (1 study, (266)) or had equivocal findings (3 studies, (260, 263, 267)). Of the two studies with the highest risk of bias, one found a positive association between air pollution exposure and ALRI (261) and the other had equivocal findings (262). Due to the variety of methodologies used for selecting participants and assessing exposures and outcomes, different aspects of different studies are likely to have biased the findings in both directions (towards and way from the null hypothesis). It is therefore difficult to establish an overall direction of bias for these studies.

3.4. Discussion

This chapter describes a synthesis of the current evidence base for the relationship between household air pollution and adult ALRI, identifying 8 eligible studies that have quantified this relationship. Despite a paucity of available studies, the available data provides some evidence for an increased risk of adult ALRI from exposure to household air pollution. The review has highlighted disparities between the findings of the studies, although these are unsurprising given the methodological heterogeneity seen. Methodological limitations were also noted, including poor exposure and outcome classifications, and potential biases including selection and recall biases. The current evidence is not sufficient to make a direct assessment of the potential impact of household air pollution on adult ALRI.

Methodological differences in exposure classification are a likely source of variation between study findings in this review. Indirect classification of exposure to household air pollution was common, and may have resulted in exposure misclassification. Accurate measurement of exposure to pollutants such as $PM_{2.5}$ is challenging in the field. Monitoring is becoming more practical, with a variety of devices now available, which will benefit future population studies of health impacts such as ALRI. Exposure, however, needs to be measured at repeated intervals to accurately classify levels for diseases with a long latency period from defined exposures (COPD/ALRI), and this remains a challenge.

With regards to major sources of bias and error, the quality of studies identified was varied. Two studies used retrospective records of patients, and none of the prospective studies reported response or refusal rates of participants, so may have been subject to selection bias. Several studies did use some form of random sampling (although details were scarce), but the cross-sectional study from Serbia only recruited women who attended a health centre so the findings are not generalisable to the wider

population. The case-control study from Mexico used patients with otolaryngeal disease as controls, but as household air pollution can also be a risk factor for this outcome (2), this may have biased the findings towards the null. Only one study directly measured exposure to household pollutants. All other studies relied on questionnaire responses regarding exposures, which are prone to recall bias, with varying recall periods and often poor definitions of exposure. Similarly, several studies relied on potentially biased and inaccurate recall of previous illness; outcome misclassification may have diluted the true effect. Three studies used prospective assessment of patients by a health-care worker for the outcome classification, although the quality of definitions used varied. Three studies made no adjustment for confounders, and other studies failed to adjust for some important confounders such as smoking, and so their findings may be misleading. Four studies were cross-sectional and are therefore unable to establish temporality between exposure and outcome.

Although the literature was previously reviewed as part of the Comparative Risk Assessment conducted for the Global Burden of Disease study 2010 (10), this review provides an update, adding 5 studies to the evaluation. This review also provides a narrative summary of the current evidence which was not previously available, and a critical appraisal of methodological limitations. The review included a comprehensive search of published bibliographic databases - including databases from Africa and Latin America - and available grey literature, including contact with known researchers in the field undertaking relevant research. The review could have been enhanced with appraisal of Chinese language databases, as a lot of relevant research has been undertaken in relation to the Chinese National Stove Programme, however this was not practical. Due to the paucity of evidence, publication bias was not assessed.

Diseases associated with household air pollution disproportionately affect those living in poverty, who rely on solid fuels to meet their energy needs (currently 3 billion people (268)). To consolidate Global Burden of Disease study estimates - which currently use modelled data from other sources of air pollution - we have attempted to quantify the association between household air pollution and adult ALRI. Whilst it is likely that the burden of disease from household air pollution in adults includes ALRI, we have not been able to confirm this association based on the current evidence. Prospective research, using robust direct exposure measurement and accurate classification of outcome with adjustment for confounders, is required to improve the evidence base. However, this is difficult to achieve in the challenging and resource-limited environments in low and middle income countries where exposure to household air pollution is mostly seen. Chapters 4 and 5 further explore the methodological challenges

associated with conducting such studies in resource-poor settings, with a focus on developing tools to improve exposure assessments.

4. Exploring airway macrophage particulate load from induced sputum as a potential biomarker of air pollution exposure

4.1. Introduction

In Chapter 3 we presented the findings of a systematic review of the literature on the association between household air pollution and ALRI in adults. Whilst we found some evidence for an association we noted important methodological limitations to the studies we included. One of these was the robust ascertainment of exposure to household air pollution. In this chapter we describe work exploring AMPL from induced sputum samples as a potential biomarker of air pollution exposure. For convenience, this work was completed in Liverpool, UK, taking advantage of pre-existing ethical approval for conducting research with respiratory patients. This study was conducted to inform the methodology for a future study of household air pollution and ALRI in adults in Malawi (presented in Chapter 6) and to inform the methodology for future trials of interventions of household air pollution reduction strategies on health outcomes.

Airway macrophages (AM) are inflammatory cells that provide a first line of immunological defence in the lungs, by ingesting and clearing foreign bodies – including particulate matter (PM) - and pathogens. The phagocytic action of AM may provide the basis for a biomarker of PM exposure. The particulate load within AM is: increased in individuals who report exposure to household air pollution compared to those who do not (171); statistically different between individuals who use different types of domestic fuel (65); and associated with exposure to outdoor PM in commuters who cycle in London (269). Correlation between AMPL and worsening lung function supports a possible pathophysiological role (270). A recent systematic review of studies calculating AMPL concluded that this biomarker is suitable for assessing personal exposure to PM, but that technical improvements are needed before this method is suitable for widespread use (271).

Measuring AMPL involves examining AM - usually obtained by inducing sputum using nebulised hypertonic saline or BAL - using light microscopy. Once cell monolayers (Cytospins^{TM}) have been obtained, several different digital image analysis software programmes can be used to calculate AMPL. ImageJ software (http://rsbweb.nih.gov/ij/, superseding a similar software, Scion Image) and Image SXM

software (272) (http://www.ImageSXM.org.uk) have both been used for this purpose and are both available in the public domain (68, 173, 269).

ImageJ has been shown to successfully identify areas of cytoplasm and PM, but requires the operator to manually adjust the threshold settings, and so is time consuming and subjective (269). The Image SXM method was developed to be less labour intensive and less subjective (68), and can automatically calculate the area of PM within a macrophage using threshold settings that are set at the beginning, allowing a batch of images to be analysed without further operator input. It is unknown whether these two methods provide comparable results, and there is no previously reported objective comparison of the feasibility of these two methods. Furthermore, whilst ImageJ has previously been used for induced sputum samples, Image SXM has only been used with BAL samples. BAL is not a feasible sampling method for use as a field biomarker, due to its invasive nature, but induced sputum potentially could be.

4.1.1. Objectives

- To establish whether Image SXM methodology can be extrapolated for use with induced sputum samples.
- To compare the particulate load results obtained using Image SXM and ImageJ.
- To provide an objective assessment of whether Image SXM and ImageJ methods are feasible – with regard to resources, expertise and time required - for use as a biomarker.

Comparison of particulate load with air pollution exposure measurements is beyond the scope of this project, and further work to establish a correlation will be required before particulate load can be used as a biomarker.

4.1.2. Contributors to the chapter

The study was devised by myself, with input from Dr Jamie Rylance (LSTM, UK), and I wrote the study protocol. Ethical approval was sought by Dr Kevin Mortimer (LSTM, UK). I was responsible for all aspects of the study implementation, including participant recruitment (with assistance from Dr Latifa Patel and Kevin Mortimer), sputum induction, sputum processing (with assistance from Dr Andy Cross, University of Liverpool, UK), sputum analysis, data management and analysis. I wrote the manuscript for publication, with editing from Jamie Rylance, Latifa Patel, Stephen Gordon (MLW, Malawi) and Kevin Mortimer. Dr Will Monteiro and Beverley Hargadon (both Glenfield Hospital, Leicester, UK) taught me how to conduct sputum induction and process sputum. Dr Rossa Brugha (Barts and the London School of Medicine and Dentistry, UK) taught me ImageJ methodology.

4.2. Methods

4.2.1. Participant involvement

Respiratory patients were recruited via outpatient respiratory clinics at Aintree University Hospital, Liverpool, UK. All consenting adults over 18 years old with asthma or bronchiectasis, who did not meet safety exclusion criteria (according to local safety guidelines for conducting spirometry based on known contraindications (273), see Box 4-1), were recruited.

Box 4-1: Safety Checklist – Exclusion Criteria for Sputum Induction

- FEV_1 < 60% / < 1.0L (post Salbutamol 200 micrograms)
- SpO_2 < 90% on room air
- Unable to take salbutamol
- Extreme shortness of breath
- Acute Respiratory Distress Syndrome
- Known haemoptysis
- Known arrhythmias/angina
- Known thoracic, abdominal or cerebral aneurysms
- Recent pneumothorax
- Pulmonary emboli
- Fractured ribs / recent chest trauma
- Recent eye surgery
- Known pleural effusions
- Pulmonary oedema
- Thrombocytopenia (Platelets < 25)

4.2.2. Sputum induction

Participants underwent sputum induction on one occasion each in August-October 2013 following standard procedures (274). Pre-procedure Salbutamol (200 micrograms) was given to prevent bronchoconstriction. Baseline spirometry was performed to European Respiratory Society and ATS standards (275) using a MicroMedical MicroLab Mk8 Spirometer (Cardinal Health UK). Three x 5mls of hypertonic saline (3%, 4%, 5% saline given in stepwise fashion, lasting up to 5 minutes per nebulisation)

were nebulised via Omron NE-U17 Ultrasonic Nebuliser (Omron Healthcare Europe) to induce sputum production. Lung function was monitored throughout the procedure to observe for bronchoconstriction. If the FEV_1 dropped by 10-20% during the procedure, induction was continued but without an increase in hypertonic saline strength. If the FEV1 dropped by more than 20% the procedure was abandoned for the participant's safety.

4.2.3. Sputum processing

Sputum samples were processed in laboratories at Aintree University Hospital, Liverpool, UK. Sputum samples were kept on ice and sputum plugs were manually extracted and treated with 0.1% Sputolysin (Merck Chemical Ltd, UK) for fifteen minutes to remove mucus. Phosphate Buffered Solution (Sigma-Aldrich, UK) was added and cells were filtered and centrifuged at 2200 revolutions per minute (rpm) for ten minutes at 4°C (Heraeus Megafuge 1.0R, ThermoFisher Scientific, USA). The pellet was re-suspended at $0.5x10^6$ cells per ml and two x 100µl of suspension was cytocentrifuged (Shandon Cytopsin 4, ThermoFisher Scientific) onto microscope slides at 450rpm for 6 minutes to produce three cytospins per participant. Slides were fixed in methanol and stored until staining. One slide per participant was stained using Hemacolor Staining kit (Merck-Millipore, Germany) for ImageJ analysis. One slide was stained using Hemacolor Solution 2 (eosin) only (dipped for 9 seconds), so that only the cytoplasm was stained (a method previously developed for optimising Image SXM analysis (68)).One slide per participant was stained with Diff-Quik (Dade Behring, Deerfield, IL, USA) for differential cell counts : 400 cells were counted per participant, using a Leica DM IL light microscope at x40 magnification, and result are reported as a percentage of the total cells seen. Cytospins with a leukocyte/squamous epithelial cell ratio of ≤5 were deemed inadequate and therefore excluded from the analysis (276).

4.2.4. Digital image acquisition

Cytospin slides for ImageJ analysis were photographed at x60 magnification using Nikon Eclipse 80i digital microscope (Nikon Instruments Europe BV) with Nikon NIS-Elements BR software; 50 macrophages were captured per participant where possible (in cases where less than 50 macrophages were present on the cytospin a reduced number was used). Slides for Image SXM analysis were imaged at x40 magnification using a Leica DM IL light microscope (Leica Microsystems UK Ltd) with a Nikon E990 digital camera (Nikon Inc., USA); where possible 50 microscope fields (with at least one macrophage per field) were captured per participant - all the macrophages captured in a field were analysed. In cases where less than 50 images from the whole cytospin contained a macrophage this reduced number of macrophages-containing images, and all macrophages within those images, were included in the

analysis. Images for both methods were taken systematically using a predefined method to prevent duplication or biased image selection, as shown in Figure 4-1.

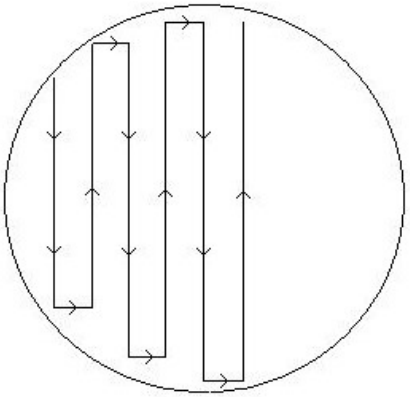

Figure 4-1: Systematic digital image acquisition.
The pathway used to acquire digital images of cytospin 'spots' is shown.

4.2.5. Image SXM analysis

Image SXM automatically calculates the area of cytoplasm within an image and the area of PM within that cytoplasm. As I intended to calculate the amount of carbon within macrophages only, other cells and debris were removed by hand from the images prior to the analysis using Adobe Photoshop Elements version 6.0, to prevent incorrect calculations of cellular and PM areas (Figure 4-2). Image SXM (version 1.92, April 2011) variable settings were optimised for cytoplasm (upper and lower size limits and density threshold) and PM (density threshold) detection by adjusting settings for a range of images from different participants. Values which consistently maximised identification of PM without increasing false positive identification were used. These settings were then applied to the analysis of all images from all participants. Fifty images per participant were analysed to generate output images (Figure 4-2) and the arithmetic mean percentage of total cellular area occupied by PM was calculated by Image SXM. The blink comparison function, which provides an overlay of images, was used to compare original and output images; subjective discordance between total cellular or PM area led to removal of that image from the analysis. Participants with fewer than ten images remaining were excluded from the analysis.

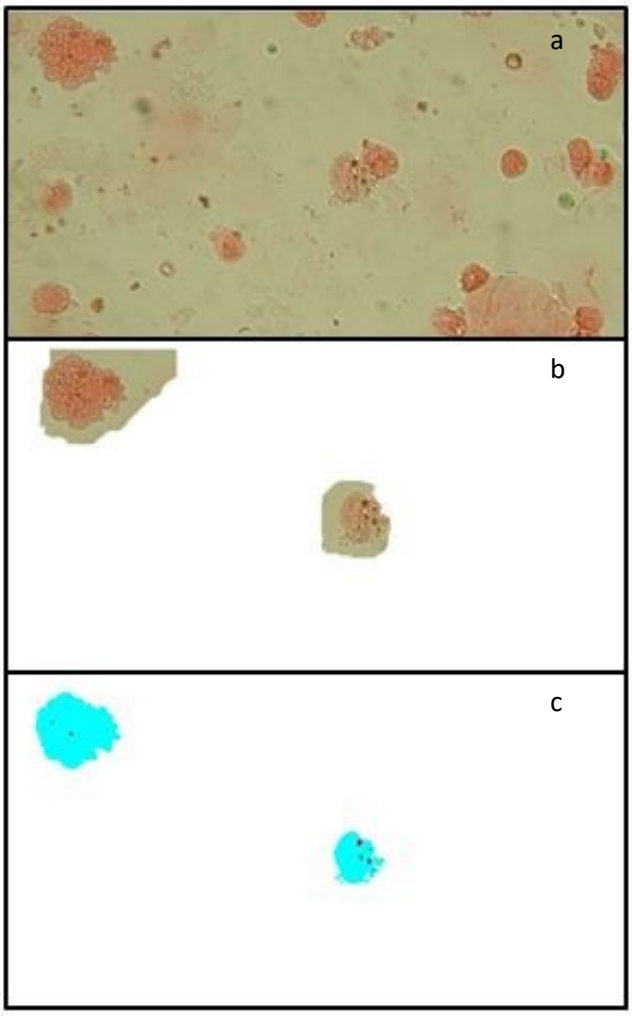

Figure 4-2: Image SXM methodology.
Digital images of the cytospins (a) were edited to remove all non-macrophage cells and debris (b). Image SXM then calculated the area of cytoplasm (blue) and particulate matter (red), mapped out in the output image (c).

4.2.6. ImageJ analysis

A stage micrometer (Agar Scientific, UK) was used to calibrate image size. Colour images were converted to 32-bit black and white images using ImageJ (version 1.46r). The "threshold" settings were manually adjusted to obtain the best fit of red over black areas (264) (Figure 4-3). The freehand select function was used to select PM (Figure 4-3) that was within the cell, and to exclude red areas other than PM, such as nucleus. ImageJ calculated the area of PM within the selection. Thresholds were adjusted to obtain the best fit for different particle aggregates in each macrophage. The median area from 50 macrophages was calculated. This methodology is a refinement of previously used techniques (269),

adapted from earlier Scion Image methodology (171). Since publication of this methodology, the technique has been refined by the same research group and no longer requires editing of images to remove the nucleus, as the same results can be achieved by using the freehand select function to select the particles (personal correspondence, Dr R. Brugha, Barts and the London School of Medicine and Dentistry).

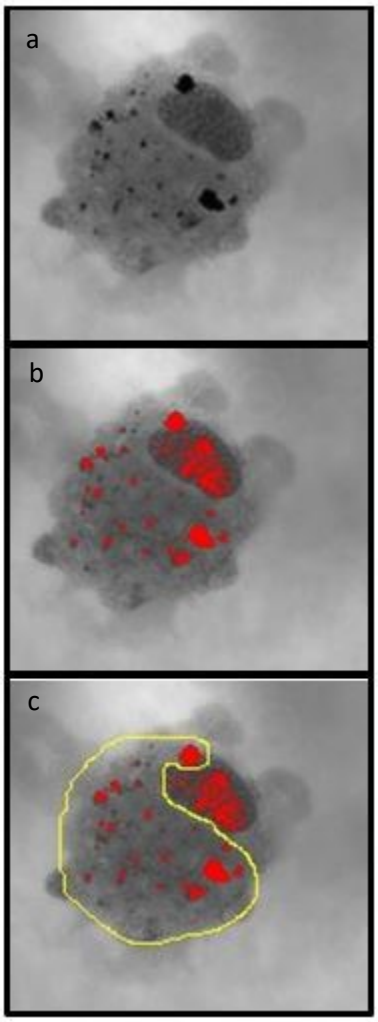

Figure 4-3: ImageJ methodology.
The threshold level was adjusted until the areas black of particulate matter seen in the original image (a) turned red (b). The particulate matter was then selected using the freehand selection function (c).

4.2.7. Feasibility comparison of methods

The time taken for image capture and analysis of the final 11 samples was recorded, along with an inventory of the required equipment and expertise for each method. The analyses for the first two participants were not timed, to prevent inaccurate results whilst becoming familiar with the software programmes.

4.2.8. Statistical analysis

Data was analysed using SPSS v21. As Image SXM calculates the mean proportion of cytoplasm occupied by PM in 50 macrophages, whereas ImageJ calculates the area of PM (in µm2) per macrophage, the results cannot be directly compared. Results for each method were therefore ranked according to particulate load, and the rank order was compared using a Spearman Rank Order Correlation test. Participant characteristics were compared using Chi-square and Mann-Whitney U tests. Time taken to conduct the analyses was compared by Wilcoxon Signed Rank test. A p value of <0.05 was considered statistically significant.

4.2.9. Ethical approval

The East Midlands – Derby 1 Research Ethics Committee (REC)[2] approved this work (REC reference: 11/EM/0269). Written informed consent was obtained from all participants.

4.3. Results

4.3.1. Sputum induction

21 participants were recruited and attended for sputum induction and 1 participant was excluded due to baseline hypoxia (28 other recruited participants failed to attend). Of 20 participants undergoing sputum induction, samples were successfully obtained from 19 (Figure 4-4). No adverse events occurred. Cytospins from six (32%) participants were inadequate due to their leukocyte/squamous epithelial cell ratio. The characteristics of the 13 participants who provided an adequate sample are shown in Table 4-1. There was no significant difference in characteristics between those who provided an adequate sample and those who did not (data not shown). The differential cell counts are shown in Table 4-2.

[2] Approval was awarded by a REC outside of Liverpool due to a system in which the next available REC in the NHS Health Research Authority reviews applications for ethical approval.

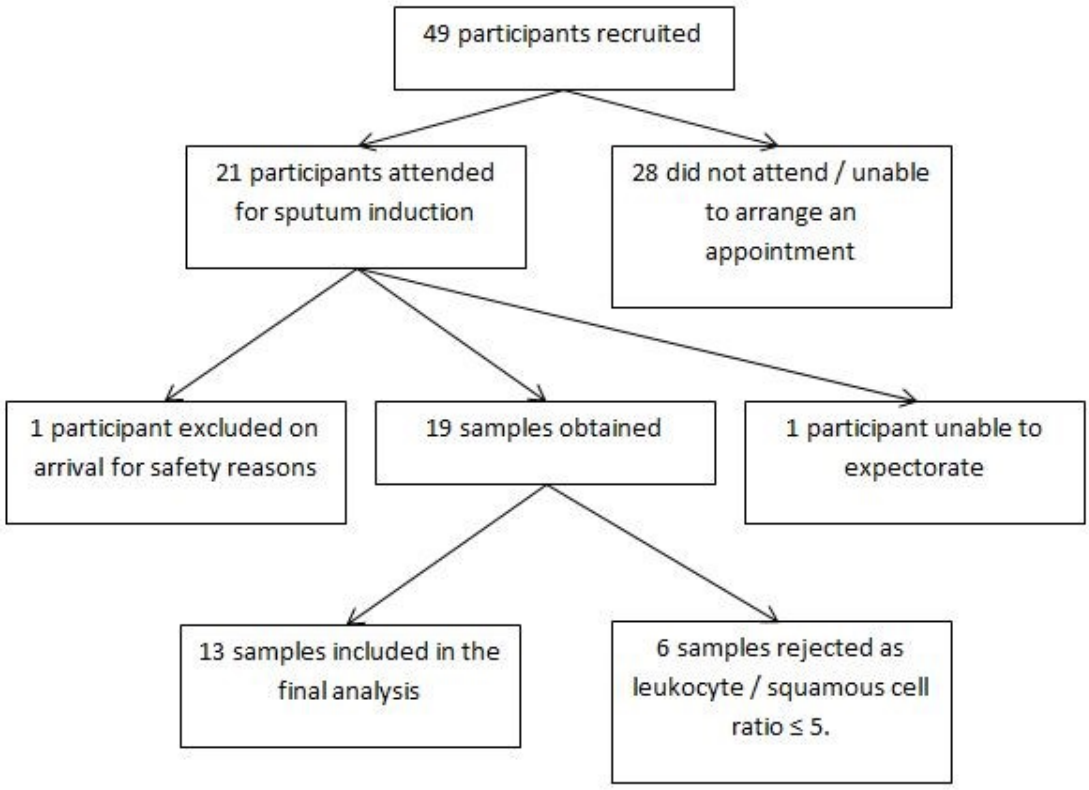

Figure 4-4: Participants and samples.
The flow chart shows the number of consented and recruited patients, and how many samples were obtained and included in the final analysis.

4.3.2. Feasibility of methodology

Median time for analysis of each participant was significantly lower for ImageJ (26 mins, interquartile range (IQR): 21-30) than for Image SXM (54 mins, IQR: 43-68), p=0.008. Including the time taken for image acquisition, the median time was not significantly different between ImageJ (51 mins, IQR: 46-65 mins) and Image SXM (66 mins, IQR: 59 – 84), p = 0.424. For the Image SXM method, 58% of the 'analysis time' was spent editing the images prior to analysis: editing the images to remove non-macrophage cells and debris using Adobe Photoshop Elements was necessary (a step not required when using BAL samples).

Table 4-1: Characteristics of 13 participants.

Participant characteristic		
Gender	Male, n (%)	9 (69)
	Female, n (%)	4 (31)
Age	Median (IQR)	57 (39-67)
Respiratory diagnosis	Asthma, n (%)	8 (62)
	Bronchiectasis, n (%)	2 (15)
	Both, n (%)	3 (23)
Smoking status	Never smoked	8 (62)
	Ex-smoker	5 (38)
Spirometry	FEV_1, median (IQR), litres	1.80 (1.47-2.26)
	FEV_1 % Predicted*, median (IQR)	73.5 (60.1 - 77.6)
	FVC, median (IQR), litres	2.8 (2.47 – 3.82)
	FVC % predicted*, median (IQR)	91.2 (87.6 – 109.0)

*Normal values: FEV_1 % Predicted and FVC % Predicted: ≥80%

IQR: Interquartile Range; FEV_1: Forced Expiratory Volume in 1 second; FVC: Forced Vital Capacity.

Table 4-2: Differential cell counts.

Cell type	Cell count (%) (Median (IQR) of 13 participants)
Neutrophil	72.5 (51.1-90.1)
Macrophage	10.0 (4.1-25.8)
Eosinophil	1.6 (1.0-8.5)
Lymphocyte	2.3 (1.2-3.5)
Metachromatic	0.0 (0.0-0.0)
Bronchial epithelial	2.8 (1.1-12.6)
Squamous epithelial	2.3 (0.8-6.5)

Normal values (median %): neutrophils 24.1%, macrophage 62.9%, eosinophils 0.5%, lymphocytes 1.3% **(274)**.

IQR: Interquartile Range

A mean of 49 macrophages per participant were included in the ImageJ analysis (total 632 macrophages). A mean of 43 images of per participant were captured for Image SXM analysis (total 558 images). During the Image SXM process, 72% of images were removed following the initial analysis as they were deemed to be inaccurate (either over- or under-estimating AMPL) using the blink comparison function (Figure 4-5), resulting in a further four participants being excluded from the study. The analysis was repeated with only the remaining 143 images (median 14 images (IQR 11.5-20) per participant). If only these nine participants are included, median time taken increased to 67 mins (IQR 47-72) for Image SXM analysis and 83 mins (IQR 64-87) including image acquisition time. After this second analysis, the images were visually checked again: it appeared that some images – which had been accurately analysed during the first analysis – were inaccurately analysed in the second analysis, despite threshold settings remaining the same (Figure 4-6). Further exclusion of images and repeat analysis was not done at this stage, as the process was deemed inaccurate, inconsistent and unfeasible.

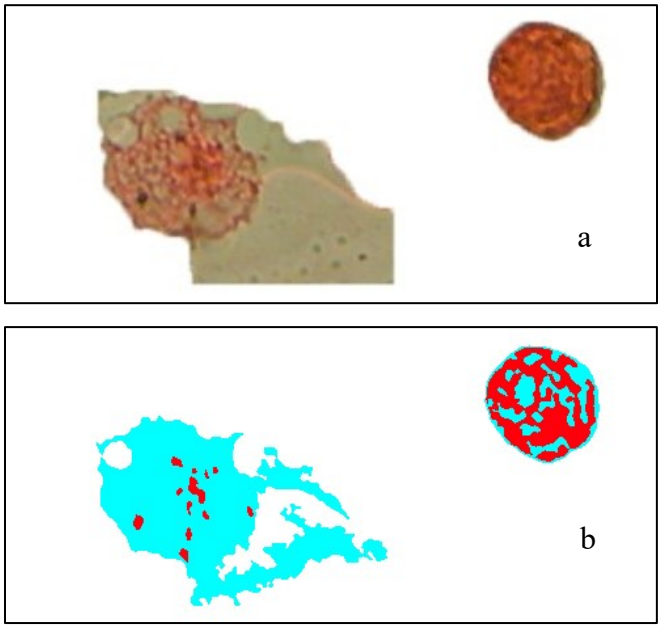

Figure 4-5: An example of inaccurate Image SXM analysis.
Comparing the original image (a) to the output image (b), the cytoplasmic area (blue) of the airway macrophage on the left has been overestimated, and the particulate matter (red) of the airway macrophage on the right has been overestimated.

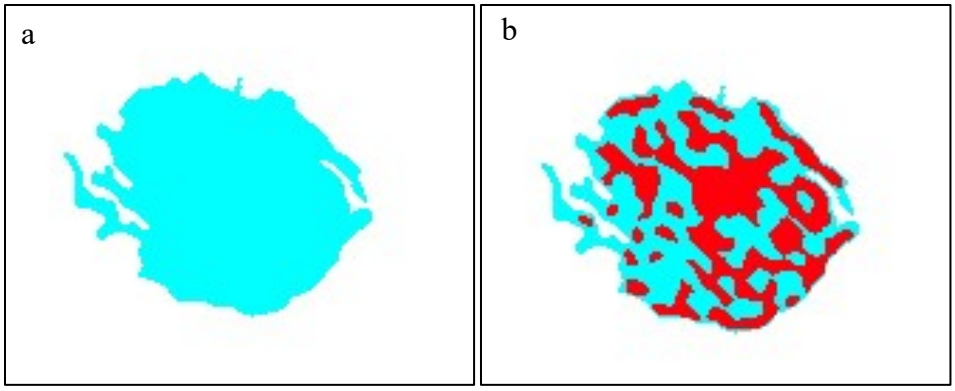

Figure 4-6: An example of inconsistent SXM analysis.
The same image of the same AM was analysed twice using the same threshold settings. The output image from the first analysis (a) is grossly different to the output image from the second analysis (b).

A comparison of the resources required for each method is shown in Table 4-3. ImageJ software can be downloaded for free for use with both Windows and Mac operating systems. Image SXM software can also be downloaded for free, but can only be used with a Mac operating system, and Adobe Photoshop Elements software must be purchased. Whilst the Image SXM process only requires a microscope (with digital image acquisition capabilities) with a x40 objective, the ImageJ process should be used with a x100 objective. In this study a x60 objective was used, as greater magnification was not available.

4.3.3. Airway macrophage particulate load

Considerable morphological heterogeneity was seen between AM, both within samples and between participants, with wide variations in AMPL (Figure 4-7). The cytoplasms of the AM in this study were noted to be granular and heterogeneous (Figure 4-7), unlike the homogenous appearance of cytoplasm seen in macrophages obtained by BAL (65).

ImageJ analysis of 13 cytospins revealed a median AMPL of 0.38µm^2 (IQR 0.17-0.72 µm^2) (Table 4-4). Image SXM analysis of 9 cytospins calculated a median total cellular area occupied by PM of 4.0% (IQR 2.3-6.0%) (Table 4-4). There was no statistically significant correlation between results obtained using the two methods (correlation coefficient = -0.42, p = 0.256).

Table 4-3: Comparison of resource requirements for methods.

Resource	Image SXM	ImageJ
Equipment required for sputum induction and sample processing	Identical specialist equipment and facilities required regardless of analysis method	
Image acquisition equipment	Microscope with x40 objective and digital image capturing capabilities	Microscope with x100 objective and digital image capturing capabilities
Analysis software availability	In the public domain – available free of charge	In the public domain – available free of charge
Additional image editing software	Purchase required	Not required
Operating system for analysis software	Compatible with Mac operating systems	Compatible with Mac and Windows operating systems
File type availability	TIFF	JPEG, TIFF, GIF, BMP, DICOM, FITS and 'raw'
Time required for sputum induction and processing	Approximately 90-120 minutes per participant	
Time required for image acquisition	15 min	27 min
Time required for image analysis (including image editing if required)	54 min	26 min

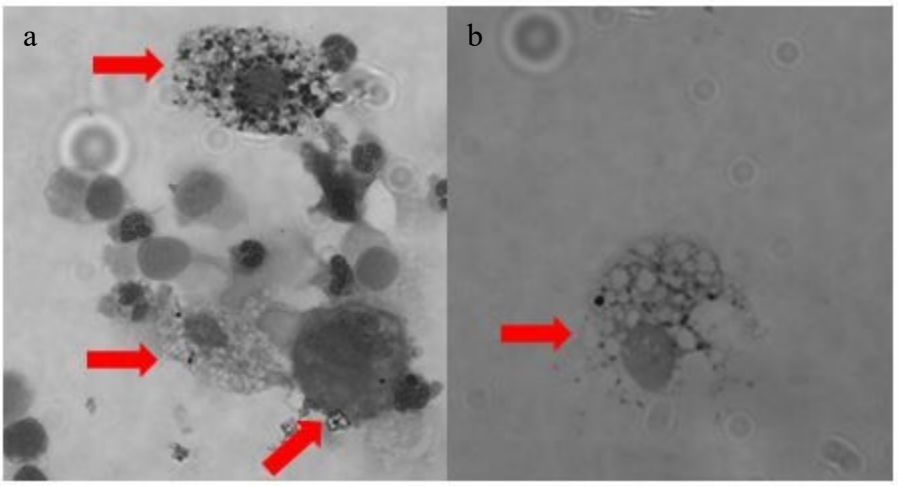

Figure 4-7: Airway macrophage heterogeneity.
The morphology of airway macrophages (shown with red arrows) was varied within the same sample (a) and between different participant samples (a & b). The particulate load also varied between macrophages in the same sample (a).

Table 4-4: Airway macrophage particulate load results using Image SXM and ImageJ methodology.

Participant	Image SXM Result Mean cytoplasmic area occupied by particulate matter, % (standard error)	ImageJ Results Median particulate load per macrophage, μm^2
1	2.5 (0.5)	0.64 (0.00 - 2.10)
2	4 (2)	0.70 (0.00 - 2.93)
3	6 (5)	0.20 (0.00 - 1.64)
4	6 (4)	0.34 (0.00 - 2.53)
5	1 (2)	0.73 (0.00 - 3.83)
6	3 (3)	0.38 (0.00 - 2.31)
7	6 (5)	0.00 (0.00 - 0.13)
8	Rejected	0.13 (0.00 - 2.47)
9	Rejected	0.33 (0.00 - 0.77)
10	11 (7)	2.41 (0.00 - 4.88)
11	2 (0.5)	2.42 (0.35 - 5.71)
12	Rejected	0.00 (0.00 - 0.79)
13	Rejected	0.63 (0.00 - 1.51)

4.4. Discussion

A biomarker which can be used in the field to assess an individual's air pollution exposure will be a valuable tool for research into the health effects and benefits of interventions. This study set out to explore the feasibility of using induced sputum samples for assessment of AMPL as a potential biomarker.

Although the procedure was well-tolerated by all participants who underwent sputum induction, there was a low appointment attendance rate despite multiple appointments being offered at their convenience. This may be due to participant's availability, but may also reflect an unwillingness to undergo the procedure suggesting that sputum induction may not be acceptable to the wider community. A third of participants were unable to produce adequate samples. These factors resulted in a small sample size, a major limitation of this study, but also reflects a potential limitation in the feasibility of using induced sputum as a biomarker.

The time taken for the Image SXM method was substantially lengthened by the need to manually edit images prior to analysis to improve accuracy. This editing is not required when using this software with BAL samples, which tend to have few other cells or debris. This negates one major perceived advantage of using Image SXM, which was previously seen to be quicker, easier and more automated than ImageJ. Despite editing images to improve quality, this study has demonstrated that Image SXM makes inaccurate assessment of the majority of images, with 72% of images needing to be removed following inspection. This substantially reduces the number of macrophages included in the analysis, thereby reducing the accuracy of the estimate for particulate load obtained.

ImageJ was the quicker method for image acquisition and analysis (median 51 minutes). Image capturing software used in this study for the ImageJ method delayed the process by approximately 15 minutes. A limitation of this study as there was that different equipment was used for acquiring images for the two methods, as there was no equipment available with both a x40 and x60 (or x100) objective. In future studies, if a simpler method for acquiring images at x60 or x100 magnification is available then this could reduce the amount of time required for the ImageJ method. However, when combined with the time taken for sputum induction and processing (usually >90 minutes), this process is unlikely to be feasible for widespread use in large studies given the total time required (>2 hours per participant).

Both methods require considerable expenditure for clinical and laboratory equipment. Previously published studies using ImageJ method report using a microscope with a x100 objective, while the Image SXM method requires a x40 objective, both with digital image acquisition capabilities. In this study a x60 objective was used for the ImageJ method, as greater magnification was not available with digital image capturing capabilities. Although this may have theoretically reduced the accuracy of the ImageJ methodology in this study, I experienced no difficulties visualising PM within the macrophages and still found ImageJ to be the more reliable of the two methods for detecting PM. As I do not comment on the accuracy of the ImageJ method in comparison to a gold standard assessment of exposure, this limitation of this study does not have a major impact on the findings. However, it does emphasise the need for specialised equipment, which has implications for feasibility.

Both software programmes are available free of charge but ImageJ is more widely compatible. Image editing software must be also purchased if using Image SXM with induced sputum. The facilities and equipment required for inducing and processing sputum are likely to preclude the use of this technique in rural or resource poor settings.

A further limitation of this study is that image capture of macrophages – which can be difficult to differentiate from other cell types (particularly on cytospins stained only with eosin for Image SXM analysis) - was only performed by one reader, with support from a senior cell biologist, without a priori criteria for inclusion. This may have resulted in incorrect identification of some cells. Independent image capture and slide analysis by two individuals with a high level of expertise may improve accuracy of macrophage identification, although this represents an additional challenge for implementing these methods in resource limited settings.

ImageJ method requires higher levels of operator training for image analysis than Image SXM, due to the subjective nature of the analysis process. Further work to assess intra- and inter-observer reliability using the ImageJ method is required before this is widely used – this was not evaluated as part of this study in which only one unblinded reader performed the analysis.

Although previously successfully used with BAL samples, Image SXM appears to not perform as well with induced sputum macrophages. This is possibly due to the heterogeneous and granular nature of these macrophages making it difficult for the software to distinguish between cytoplasm and PM, as has been observed in previous studies (271). I postulate that the difference in appearance compared to BAL macrophages is either due to these being a different population of macrophages, taken from a more proximal part of the airways, or due to cell stress or apoptosis resulting from the sputum induction process, although I did not measure cell viability in this study. Steps were taken to ensure threshold settings were optimised for this batch of images, but due to the heterogeneity seen these settings were not always optimal for each individual image. Image SXM does include an option to adjust the threshold settings manually for different images. This might improve accuracy but would make the process more time-consuming, and would not account for heterogeneity of macrophages within the same image (Figure 4-7). Optimising the threshold settings for each image might reduce the number of images discarded from Image SXM following visual checking for accuracy (Figure 4-5). This might increase the sample size and therefore the precision of estimates, although would make the process more subjective.

Of major concern is that, when the Image SXM analysis was repeated (following removal of images), the retained images often had different results to the results for the same images in the initial analysis, despite no change to the software settings. This has highlighted worrying inconsistencies with software, leading to inaccurate estimates of particulate load.

The lack of correlation observed in AMPL results between the two methods is unsurprising given some of the difficulties outlined above. To determine the accuracy of either method, comparison with an external comparator is required, such as an individual's PM exposure data. This, and assessment of intra- and inter-observer reliability, were beyond the scope of this study. An association between AMPL calculated and the number of peak exposures to PM has been demonstrated in London cyclists (277), but further exploration of this relationship in other settings is required. The results obtained by the ImageJ method in this study are comparable to that of healthy British children (0.41 μm_2 PM per macrophage) (270). Other studies using ImageJ methodology have suggested that AMPL does correlate with exposure (171, 270).

Given the fundamental role of AM in the defence against inhaled pollutants, further exploration of the relationship between AMPL and pathophysiology is an intuitive way to improve understanding of the health impacts of air pollution. Optimising digital analysis software or using alternative methods for quantifying AMPL, such as spectrophotometry, may assist with this, but is unlikely to provide a useful field biomarker of exposure.

Direct measurement of air pollution exposure is costly, logistically complicated and intrusive to the individual. Studies investigating the health impacts of air pollution exposure and the benefits of interventions are limited by the challenges associated with accurately quantifying exposure (170). A biomarker of air pollution exposure will be a useful tool to facilitate research addressing the high burden of disease associated with air pollution. This small study has not established whether AMPL is an accurate biomarker of pollution exposure, but has identified that digital analysis AMPL from induced sputum samples is unlikely to be appropriate for widespread use as a tool for large-scale clinical epidemiological and intervention studies. Priority should be given to developing a point-of-care biomarker of exposure, without the need for specialist training and equipment, to facilitate the large public health intervention trials that are urgently needed. Potential biomarkers requiring further exploration include direct measures of combustion products, such as eCO (explored further in Chapters 5 and 6), exhaled carboxyhaemoglobin, exhaled volatile organic compounds or levoglucosan and methoxyphenols in urine (168, 170, 174, 278, 279). Indirect measures of exposure in sputum, blood and urine, including markers of oxidative stress and endothelial or epithelial damage (such as 8-isoprostane, malondialdehyde, nitric oxide, or surfactant-associated protein D), may also be promising biomarkers.

5. Exploring exhaled carbon monoxide as a potential biomarker of air pollution exposure: a feasibility study for a randomised controlled trial of a cookstove intervention in Malawi

5.1. Introduction

In Chapter 4 we explored AMPL from induced sputum as a potential biomarker of air pollution exposure for use in a future study of household air pollution and ALRI in adults. We concluded that this approach would not be suitable for use on scale in future clinical epidemiological and intervention studies and that other potential biomarkers including eCO should be looked at instead. In this chapter we describe a pilot intervention study in which we evaluate the feasibility of using eCO as a biomarker of household air pollution.

5.1.1. Objectives

- To determine whether eCO is a feasible biomarker of household air pollution that can be used in Malawi.
- To determine whether research of household air pollution exposure and its health effects is feasible in Malawi.

5.1.2. Contributors to this chapter

The study was designed by Dr Kevin Mortimer (LSTM, UK) and Professor Stephen Gordon (MLW, Malawi), with input from Vincent Doyle (Concern Universal, Malawi), Conor Fox (Clioma Ltd, Malawi), Elizabeth Banda (Clioma Ltd, Malawi), Christa Roth (Fuel and Food Consultants, Germany) and Dr Sean Semple (University of Aberdeen, UK). I oversaw the set of up study activities in Malawi. James Kachidihu, Howard Bandah and Macfary Kapanga (all Concern Universal, Malawi) performed participant recruitment and data collection, under supervision of myself and Kevin Mortimer. I performed data analysis and synthesis of the feasibility report.

5.2. Methods

5.2.1. Participants and setting

Women living in Ntcheu (a rural district in central Malawi) who cooked on traditional open wood fires (Figure 5-1), but wished to purchase a Chitetezo stove (see section 5.2.2.), were invited to take part in this pilot randomised controlled trial (RCT). Community engagement meetings were held by study workers prior to commencing the study and informed written consent was subsequently obtained in private. The consent process was completed in Chichewa, the local language. Individuals were excluded if they were current smokers or lived with a smoker. Inclusion and exclusion criteria are shown in Box 5-1.

Box 5-1. Inclusion and exclusion criteria

Inclusion Criteria

- Female
- Lives in Ntcheu district
- Interested in purchasing a Chitetezo stove
- Uses an open fire

Exclusion Criteria

- Current smoker
- Lives in same household as a smoker

5.2.2. Intervention

The Chitetezo stove is a simple clay cookstove (Figure 5-1) for burning solid fuels which was chosen as the intervention in this study as it reduces fuel consumption by approximately 40% compared to a traditional “three-stone” fire (62), it is produced locally using local materials (by women’s production groups in villages) and has a low cost (approximately $2USD).

Figure 5-1: Cooking practices in rural Malawi
a) A traditional open "three-stone" wood fire; b) a wood burning clay stove (Chitetezo).

Women in the intervention group purchased a Chitetezo stove on the day of recruitment, or the following day. They were asked to stop using their traditional open wood fire and commence cooking on their Chitetezo stove with immediate effect, following instructions from the study workers regarding use and maintenance. Women in the control group were asked to continue cooking on a traditional open wood fire and were able to purchase a Chitetezo stove seven days later.

5.2.3. Randomisation

Women were individually randomised to one of two parallel groups, in a 1:1 ratio with block randomisation (block size 10), generated using a random number table. Randomisation was concealed using pre-prepared, sequentially numbered, opaque sealed envelopes.

5.2.4. Blinding

Study workers and participants were not blinded to group allocation following the randomisation process.

5.2.4.1. Primary outcome

The primary outcome of this study was the feasibility of conducting research to assess the impact of a cookstove intervention in this setting, including exploring any logistical challenges faced. Due to the exploratory nature of this study no hard outcomes were measured, but feasibility was assessed by considering details such as participant recruitment, retention rates, and data completeness. The acceptability of the research methodology to the study population was also considered.

5.2.4.2. Secondary outcomes

Given the pilot nature of this study, the performance or impact of the Chitetezo stove were not assessed but data regarding household air pollution exposure and health were collected as secondary outcomes, to provide insight into the feasibility of assessing these outcomes. Exposure outcomes included eCO levels (potential biomarker of exposure), household $PM_{2.5}$ (particulate matter <2.5µm diameter) levels and ambulatory CO levels. Health outcomes included symptom burden (including respiratory, ocular, backache and headache symptoms in the preceding seven days) and oxygen saturations (SpO_2). Secondary outcomes were measured at baseline and seven days.

5.2.5. Data collection

5.2.5.1. Household air pollution exposure assessments

Handheld CareFusion MicroCO meters were used to assess eCO levels as a single measurement at both baseline and Day 7 assessments.

Pollution exposure monitoring was performed for a minority of women (limited by the number of monitors available) at baseline and Day 7 assessments. A TSI SidePak™ Personal Aerosol Monitor AM510, used to measure $PM_{2.5}$ in real time, was placed in a woman's home for 24 hours. Personal CO monitors (Lascar USB Dataloggers), which clipped to clothes, were worn continuously for 24 hours.

5.2.5.2. Health assessments

Questionnaire data were collected in the villages using a paper based case report form then collated in an Excel spreadsheet by a study worker. SpO_2 was measured using finger pulse oximeters.

5.2.6. Sample Size

A convenience sample of 50 participants (including both groups) was chosen based on the requirement to assess feasibility of study methodology, rather than to detect any clinical difference between the two groups.

5.2.7. Compensation

Women were compensated for their inconvenience with a gift to the value of approximately $2 USD. This gift varied between villages, depending on what was agreed prior to the recruitment process, and included either Pigeon Pea seeds or a Chitetezo stove.

5.2.8. Statistical Methods and Analysis

SPSS Statistics 19 was used to analyse data. Histograms were reviewed to assess data distribution. Chi-square tests or Fishers exact tests for comparison of categorical data between the two groups, and Mann Whitney U-tests for continuous data. A p-value of <0.05 was considered to be statistically significant. Missing data were excluded from the analysis on a case-by-case basis for health and exposure outcomes. Feasibility and acceptability aspects were reviewed through dialogue with all members of the study group during and after completion of the study.

5.2.9. Ethical Approval

This work was carried out through collaboration between LSTM, Concern Universal (www.concernuniversal.org), and Clioma Ltd (Malawi registered consultancy company). Staff from Concern Universal provided the study group with access to these communities, and approval of village elders was sought. The study received ethical approval from the LSTM REC (11.74) and the College of Medicine REC (University of Malawi) (P.07/11/1103). The study was registered with the Pan African Clinical Trials Registry (PACTR201110000324321).

5.3. Results

5.3.1. Recruitment

The study took place in November-December 2011. All women approached were keen to participate. Two women were not eligible for participation due to exclusion criteria (Figure 5-2). Following assessment by 2 study workers, one elderly woman was deemed unable to give informed consent due to a lack of understanding. Fifty-one women were recruited from 5 villages and recruitment stopped once the desired sample size had been reached. No adverse events occurred during the study. Although all of the recruited women wished to purchase a Chitetezo stove, many were unable to afford this on the day of the visit – therefore many opted to have a Chitetezo stove as their compensation gift, in order to allow them to participate.

5.3.2. Randomisation

26 women (51%) were randomised to the control group and 25 (49%) to the intervention group (Figure 5-2). There were no objections raised to the randomisation process or to a delay in receiving a Chitetezo stove.

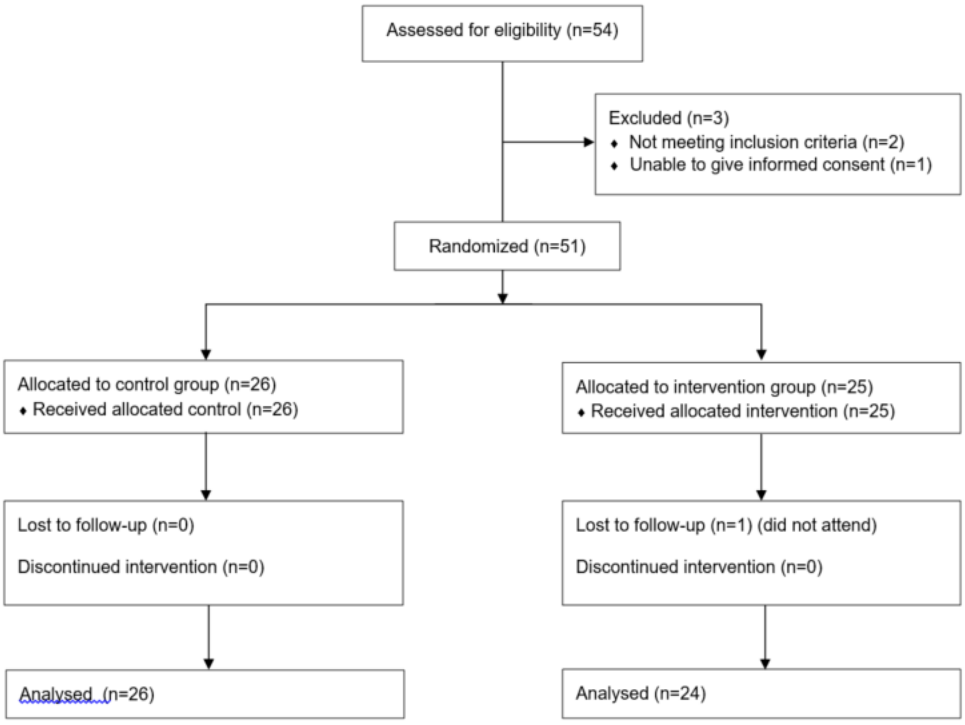

Figure 5-2: Consort flow diagram showing recruitment to the study, randomisation and loss to follow up.

5.3.3. Retention

50 women (98%) completed the main study – one woman from the intervention group did not attend the follow-up session.

5.3.4. Baseline data

As shown in Table 5-1, limited resources were available to the participants. Sources of household air pollution exposure are reported in Table 5-2. Almost all participants (98%) used wood as their primary fuel source and most cooked inside a building separate from their main house in both dry and wet seasons. The majority of women did not use any form of heating. Simple battery powered light-emitting diode lights were used by 90% of women. All of the women denied any other forms of smoke exposure.

Table 5-1: Baseline characteristics of study participants.

Parameter	Control Group (n=26)		Intervention Group (n=25)		Total (n=51)
	n (%)	Missing data n (%)	n (%)	Missing data n (%)	n (%)
Age – median [IQR]	36.5 [36.0]	2 (7.7)	33.0 [18.0]	0 (0)	38.1 [15.5]
Number of rooms in household - mean ± STD	2.5 ± 0.9	0	2.6 ± 1.0	0	2.5 ± 0.9
Number of adults in household - mean ± STD	1.8 ± 0.4	2 (7.7)	2.2 ± 0.6	1 (4.0)	2.0 ± 0.5
Number of children in house – median [IQR]	1.5 [2.0]	2 (7.7)	2.0 [2.0]	1 (4.0)	1.9 [1.6]
Roof type		0		0	
Corrugated	21 (80.8)		22 (88.0)		43 (84.3)
Grass	5 (19.2)		3 (12.0)		8 (15.7)
Window type		2 (7.7)		2 (8.0)	
No window	6 (25.0)		2 (8.7)		8 (17.0)
Space only	10 (41.7)		12 (52.2)		22 (46.8)
Glass	8 (33.3)		9 (39.9)		17 (36.2)
Water supply type		0		0	
Communal pipe	5 (19.2)		7 (28.0)		12 (23.5)
Well / bore hole	8 (30.8)		10 (40.0)		18 (35.3)
River / other	1 (3.8)		0 (0)		1 (2.0)
Communal pipe & well or bore hole	12 (46.2)		8 (32.0)		20 (39.2)
Owned by a member of the household		0		0	
Car	0 (0)		0 (0)		0 (0)
Motorcycle	0 (0)		0 (0)		0 (0)
Bicycle	10 (38.5)		11 (44.0)		21 (41.2)
Radio	7 (26.9)		11 (44.0)		18 (35.3)
Refrigerator	0 (0)		0 (0)		0 (0)
Television	1 (3.8)		0 (0)		1 (2.0)
Telephone	5 (19.2)		7 (28.0)		12 (23.5)
Computer	0 (0)		0 (0)		0 (0)

IQR = interquartile range; STD = standard deviation

Table 5-2: Participant household air pollution exposure at baseline.

Parameter	Control Group (n=26)		Intervention Group (n=25)	
	n (%)	Missing data n (%)	n (%)	Missing data n (%)
Primary cooking method		1 (3.8)		1 (4.0)
Wood	24 (96.0)		24 (100.0)	
Mbaula*	1 (4.0)		0	
Secondary cooking method		1 (3.8)		1 (4.0)
None used	21 (84.0)		24 (100.0)	
Wood fire	1 (4.0)		0	
Mbaula*	2 (8.0)		0	
Crop residue fire	1 (4.0)		0	
Dry season primary cooking location		1 (3.8)		0
Inside main living area	0		1 (4.0)	
Elsewhere inside main house	2 (8.0)		2 (8.0)	
Separate building	17 (68.0)		21 (84.0)	
Outside	6 (24.0)		1 (4.0)	
Wet season primary cooking location		1 (3.8)		0
Inside main living area	0		1 (4.0)	
Elsewhere inside main house	2 (8.0)		2 (8.0)	
Separate building	17 (68.0)		21 (84.0)	
Outside	6 (24.0)		1 (4.0)	
Primary heating method		0		0
No heating used	21 (80.8)		21 (84.0)	
Wood fire	3 (11.5)		4 (16.0)	
Mbaula*	2 (7.7)		0	
Primary lighting method		0		3 (12.0)
No lighting used	2 (7.7)		0	
Paraffin / kerosene lamp	2 (7.7)		0	
Battery powered torch	21 (80.8)		22 (100.0)	
Hurricane lamp	1 (3.8)		0	
Exposure to other smoke sources		1 (3.8)		0
None	25 (100.0)		25 (100.0)	

*Traditional stove.

5.3.5. Exposure measurements

No problems with measuring eCO were encountered: participants had no objections or difficulties performing the test correctly, and we experienced no faults with the equipment. Median eCO was 2 (IQR 2) and 3 (IQR 2) for the control and intervention group at baseline respectively. At follow-up, median eCO was 3 (IQR 2) and 2 (IQR 1) for these groups respectively. The median change in eCO from baseline to follow-up was significantly different between the two groups (median change in eCO 0.0ppm (IQR 3) and -0.5ppm (IQR 3) for the control group and intervention respectively, p=0.035).

Four women (two from each study group) wore personal CO monitors for 24 hours at baseline and peaks of up to 150ppm were detected (

Figure 5-3). One of these women (control group) declined to wear the CO monitor at follow-up due to beliefs regarding the monitor removing oxygen from the air. The results for the remaining three women at follow-up are shown in

Figure 5-3. One static PM monitor was placed in the home of a participant for 24 hours at baseline and follow-up (data not shown). It was

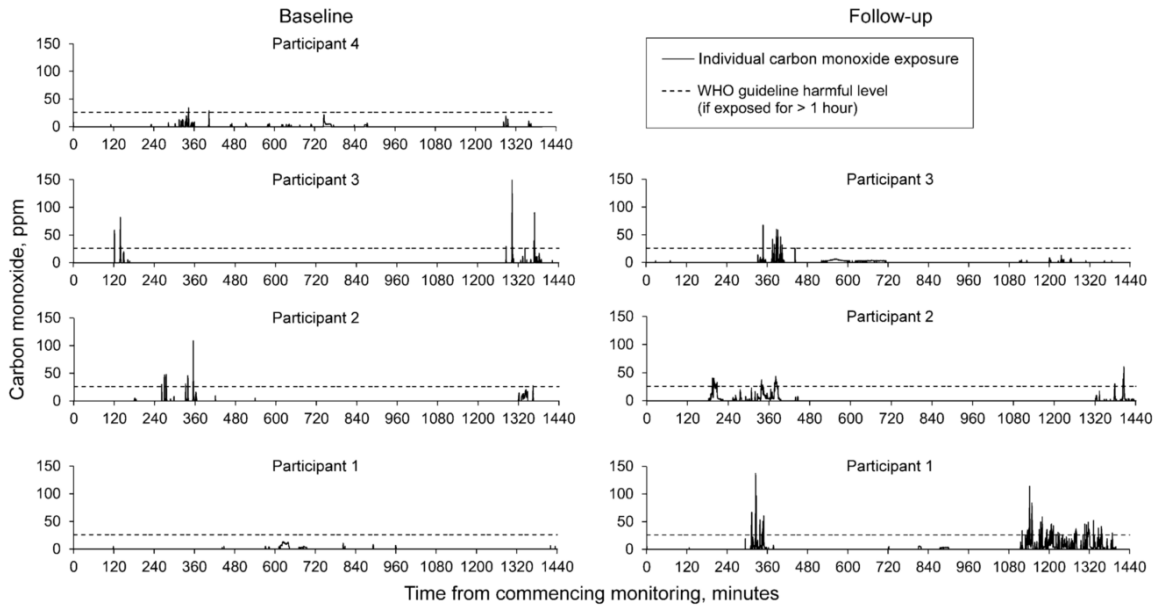

Figure 5-3: Personal exposures to CO at baseline and at follow-up in four participants.
These graphs show the detected level of ambient CO over two separate 24-h periods, measured by personal CO monitors (Lascar USB Dataloggers), which clipped to the clothes of four participants at baseline (left) and three participants at 7-day follow-up (right). A peak reading of 150ppm was detected. The black dotted line indicates the level the WHO considers unsafe if exposed for >1 hour (26ppm). Participants 1 & 2 were in the intervention group, participants 3 & 4 were in the control group. WHO = World Health Organization; ppm = parts per million; CO = carbon monoxide.

not possible to leave the monitor in the separate building where this woman cooked due to security concerns so it was instead placed inside her main living area (where there was no fire). National fuel shortages during the study period resulted in logistical difficulties relating to delivery and collection of air pollution monitors to the rural areas.

5.3.6. Symptom Burden and Oxygen Saturations

All of the recruited women completed the questionnaire, with no objections raised to any of the questions asked. Headache, back pain and cough were the most commonly reported symptoms at baseline (Table 5-3). Of those reporting a headache at baseline, the median number of days that headache had been experienced in the seven days preceding baseline was 3 (IQR 2-4) with those reporting back pain having experienced it for median 5 days (IQR 2-7). Symptom burden at follow-up was very similar (Table 5-3). There was no significant difference in change in symptoms over the seven day period between the two groups (data not shown).

No problems with measuring SpO2 were encountered. Median SpO2 when breathing room air was 98% and 99% in the control group and intervention group at baseline respectively, and 99% in both groups at follow-up.

5.4. Discussion

This study demonstrated that, despite the significant logistical challenges faced, it is feasible to conduct clinical epidemiological and intervention studies addressing household air pollution in Malawi and that eCO is an appropriate methodology for use in this context. The exploratory nature of this study, using a convenience sample, does not provide objective evidence regarding the effects of the intervention, but provides important insights into the feasibility of the research methods used which can inform future studies. The high level of interest observed in participating villages, including village elders, suggests that larger studies will be possible. Recruitment and retention rates over this short study period were excellent. The main findings of this feasibility study were that the methodologies used, including

randomisation, a short delay in receiving the cookstove, eCO measurement, household and ambulatory air pollution exposure monitoring, personal health questionnaires and SpO2 measurement were acceptable to the participants. As the compensation gift given to participants was approximately the same value as the cost of the cookstove ($2), we are unable to comment on the willingness of individuals to purchase a stove, but this was not an objective of the study. Difficulties relating to superstitious beliefs were encountered with a minority of women. Larger studies will require careful community sensitisation to address superstitious beliefs and achieve excellent retention rates.

Table 5-3: Symptoms reported at baseline and follow-up.

Symptom	Control Group		Intervention Group		*P* value
	n (%)	Missing data n (%)	n (%)	Missing data n (%)	
Baseline					
Cough	9 (34.6)	0	7 (28.0)	0	0.611*
Mucus	2 (7.7)	0	0 (0)	0	0.490†
Shortness of Breath	2 (7.7)	0	3 (12.0)	0	0.668†
Wheezing or whistling in chest	1 (4.0)	1 (3.8)	1 (4.0)	0	1.000†
Sneezing or runny nose	7 (28.0)	1 (3.8)	2 (8.3)	1 (4.0)	0.138†
Headache	12 (46.2)	0	13 (54.2)	1 (4.0)	0.571*
Burning or watery eyes	6 (23.1)	0	3 (12.0)	0	0.465†
Back pain	12 (46.2)	0	5 (20.8)	1 (4.0)	0.059*
Burns	2 (8.3)	2 (7.7)	1 (4.3)	2 (8.0)	1.000†
Family member with burns	2 (7.7)	0	1 (4.0)	0	1.000†
Follow-up					
Cough	5 (19.2)	0	5 (20.8)	1 (4.0)	1.000†
Mucus	2 (7.7)	0	1 (4.2)	1 (4.0)	1.000†
Shortness of Breath	1 (3.8)	0	0 (0)	1 (4.0)	1.000†
Wheezing or whistling in chest	1 (4.0)	1 (3.8)	0 (0)	1 (4.0)	1.000†
Sneezing or runny nose	3 (11.5)	0	5 (20.8)	1 (4.0)	0.456†
Headache	8 (30.8)	0	6 (25.0)	1 (4.0)	0.650*
Burning or watery eyes	6 (23.1)	0	1 (4.2)	1 (4.0)	0.100†
Back pain	6 (24.0)	1 (3.8)	2 (8.3)	1 (4.0)	0.247†
Burns	5 (19.2)	0	3 (12.5)	1 (4.0)	0.704†

Family member with burns	0 (0)	0	0 (0)	1 (4.0)	-

*The statistical difference between the two groups for 'Number of individuals reporting symptom' was tested using the Pearson's χ^2 test; †The statistical difference between the two groups for 'Number of individuals reporting symptom' was tested using the Fisher's exact test.

Although only a brief snapshot of exposure has been captured by the present study; CO peak levels of 150ppm detected in these households suggest concerning levels of household air pollution exposure. The WHO Air Quality Guidelines recommend that individuals should not be exposed to CO concentrations of greater that 26ppm for more than one hour (shown in

Figure 5-3) or to concentrations exceeding 87ppm for more than 15 minutes (280). Due to the small sample size, it is not possible to make statistical comparison between the before and after exposure monitoring, but the high levels of pollution seen even after introduction of the intervention may be a result of inadequate emissions reduction by the cookstove or individuals using more than one stove or fuel type in order to meet their needs, a concept known as fuel stacking (2). Air pollution exposure monitoring was logistically challenging, requiring additional visits to retrieve the equipment. In a resource-poor setting, this significant additional demand on transport and fuel resources should be considered when planning larger studies. Of note, there was a national fuel shortage at the time of this study, resulting in additional logistical challenges for the study team. Security of study equipment should also be considered, particularly with respect to air pollution monitors, as security concerns may dissuade participants from consenting to household monitoring.

Previous studies have used intensive methods of monitoring PM and CO, at individual and household levels (3, 163). However, these techniques are expensive, logistically challenging, burdensome for the participant and interpretation of results is not standardised. In order to effectively evaluate the impact of household air pollution reduction strategies, a standardised and convenient approach to assessing household air pollution exposure is required. Development of a biomarker that is representative of household air pollution exposure over the preceding weeks or months could remove the need for complex air sampling, and aid timely delivery of effective interventions to market. Several potential biomarkers have been explored but none are suitable for routine use yet (167, 281, 282). In this study, measurement of eCO was trialled and although the majority of readings were within the normal range, a significant reduction was seen at follow up in the intervention group. Small changes in eCO may be a

sensitive and responsive marker of exposure if a larger sample size were tested. Furthermore, in this study the eCO measurements were taken outdoors away from the fires/stoves; measuring eCO in the vicinity of smoke exposure may improve the sensitivity of eCO as a biomarker.

Further studies exploring household air pollution exposures and their impact on health are needed. Although the complex nature of exposure means that such studies are challenging, the findings here suggest that a larger scale study will be feasible and acceptable in Malawi. Adequate community sensitisation and careful consideration of appropriate outcome measures will be required. Intensive monitoring of household air pollution exposure levels or development of a biomarker of exposure is warranted, but significant investment will be required to achieve this. This study suggests that measurement of eCO would be worthwhile in a larger study, as a biomarker of exposure. Findings from this pilot study informed the design of two larger studies of air pollution in Malawi: the Cooking and Pneumonia Study (www.capstudy.org, (143)) and the AIR Study (described in Chapter 6).

6. Household air pollution, chronic respiratory disease and pneumonia in Malawian adults: a case-control study

6.1. Introduction

This chapter describes a case-control study, the Acute Infection of the Respiratory tract (AIR) study, which set out to test the hypothesis that household air pollution is associated with an increased risk of pneumonia in adults living in urban Malawi. This follows from the systematic review of the literature described in chapter 3 that identified a gap in the literature regarding the association between household air pollution and ALRI in adults (283) and it was informed by the methodological development work described in chapters 4 and 5. The AIR study provided opportunities to explore other potential risk factors for pneumonia, including CRD, socioeconomic status, crowding, malnutrition and alcohol intake alongside the main focus on household air pollution.

6.1.1. Objectives

- To determine whether household air pollution is a risk factor for pneumonia in urban Malawian adults.
- To determine whether CRD is a risk factor for pneumonia in urban Malawian adults.
- To explore other potential risk factors for pneumonia in urban Malawian adults.
- To determine whether eCO is an accurate biomarker of household air pollution exposure.
- To describe air pollution exposure in urban Malawi.
- To describe CRD in urban Malawian adults.

6.1.2. Integration with concurrent studies

The AIR study was integrated with other studies of adult respiratory infection which were operating concurrently at QECH and MLW at the time of recruitment, in order to maximize research efficiency whilst minimizing the burden to participants. The Burden and Severity of HIV-associated Influenza (BASH-FLU) Study (Principal Investigator: Dr Antonia Ho) aimed to establish the association between HIV infection and influenza disease severity using a case-control design. The Malawian Adult Lower Respiratory Tract Infection Severity, Aetiology and Outcome (MARISO) study (Principal Investigator: Dr Stephen Aston) was a prospective observational study nested within the Burden and Severity of HIV-associated Influenza (BASH-FLU) study, aiming to describe the clinical features aetiology and outcome of community acquired pneumonia in Malawian adults. A surveillance programme of severe acute

respiratory infection (SARI) (Principal Investigators: Dr Dean Everett and Dr Ingrid Peterson) was also in place at QECH, describing the local epidemiology of respiratory viruses. All of these studies were already operational at the time of commencing recruitment for the AIR study.

The case definition for the AIR study was aligned with the case definitions for the BASH-FLU, MARISO and surveillance studies, meaning that it was possible to co-recruit patients to multiple studies. Individuals meeting the case definitions were invited to participate in the studies they were eligible for, but were under no obligation to participate in all studies. A separate consent process was conducted for those wishing to participate in the AIR study. The recruitment and clinical assessments (using integrated data collection systems) were conducted by an integrated respiratory infection clinical research study team, and study data (including clinical specimen results) were shared between the studies if consent was given by the participant.

6.1.3. Contributors to this chapter

The study concept and design was conceived by myself, Dr Kevin Mortimer (LSTM, UK), Professor Stephen Gordon (MLW, Malawi) and Dr Ingrid Peterson (MLW, Malawi), with input from Professor Robert Heyderman (formerly MLW, Malawi) and statistical advice from Professor Brian Faragher (LSTM, UK). Research operations within QECH were supported by Dr Jane Mallewa and Dr Mulinda Nyirenda, and in the community by Dr Medson Matchaya (Blantyre District Health Officer). I coordinated study set-up and execution, with assistance from Dr Ho (BASH-FLU Study) and Dr Aston (MARISO Study).

Community control recruitment and field logistics were conducted by Chimwemwe Kambudzi, Malumbo Ng'oma, Patrick Munthali, Gift Sagawa, Stain Nkata and Paul Yonah. Community follow up appointments were conducted by Beatrice Chinoko. Hospital recruitment and assessments were conducted by the integrated respiratory infection clinical research study team (including Collins Chiliwawa, Blessings Mkwaila, Dan Chunda, Sitithana Muyaso, Rosaleen Ng'oma, Emily Lifa, Hannah Masangwi, Wezi Chimang'anga and Tiwonge Chinunda). I oversaw all community field work and the integrated hospital study team were jointly supervised by myself, Dr Ho and Dr Aston (until BASH-FLU and MARISO studies completed in January 2015). In the final months of the study, Dr Newton Kalata (MLW, Malawi) assisted with supervision of both the community and hospital study teams.

Augustine Choko (MLW, Malawi) provided support for mapping and geo-spatial aspects of the study. Chest x-rays were photographed and interpreted by myself, Dr Ho, Dr Aston or Dr Kalata. Spirometry quality assurance and interpretation was conducted independently by myself and Lindsay Zurba

(Spirometry Training Services Africa CC, South Africa). Laboratory testing of clinical samples was supervised by Brigitte Denis and George Selemani (MLW, Malawi) and tuberculosis diagnostics were supervised by Aaron Mdolo (MLW, Malawi). Assistance with operating and interpreting Aprovecho Indoor Air Pollution monitors was provided by Samuel Bentson and Alex Siedel (Aprovecho Research Centre, Oregon, USA). Support with operating University of California, Berkeley Particle and Temperature Sensor (UCB-PATS) monitors was provided by Charity Garland and Maneet Kaur (Berkeley Air Monitoring Group, University of California Berkeley, USA). Clemens Masesa (MLW, Malawi) and Rachel Lloyd (LSTM, UK) provided data management assistance. We thank the Burden of Obstructive Lung Disease (BOLD) Study coordinating centre (www.boldstudy.org) for providing permission to use BOLD questionnaires.

Dr Nico Nagelkerke (formerly MLW, Malawi) performed the interim analysis. I performed the final analysis with support from Dr Emanuele Giorgi (Lancaster University, UK), except for the geospatial analysis and data imputation using spatial interpolation which was conducted by Dr Giorgi. Dr Peterson performed the principal components analysis to construct the socioeconomic score.

6.2. Methods

6.2.1. Study design and setting

A case-control study set in Blantyre, Malawi with all cases being recruited from QECH and all controls recruited from within the city limits. HIV-positive and HIV-negative sub-groups were analysed separately, owing to anticipated lack of statistical power to explore interactions between HIV (a known major risk factor for pneumonia) and other potential risk factors for pneumonia. Furthermore, the risk factor profile for pneumonia is potentially different between individuals with HIV infection and those without, and therefore a combined analysis could be difficult to interpret.

6.2.2. Participants

6.2.2.1. Cases

Cases were defined by the presence of radiologically confirmed pneumonia requiring hospitalisation. All adult admissions to QECH were screened by study clinical officers. All medical patients admitted with respiratory symptoms were evaluated against inclusion and exclusion criteria (see Box 6-1). Due to anticipated delays in acquiring chest x-rays for patients at QECH, it was possible for a patient to be

recruited without a chest x-ray, and they were subsequently excluded if an x-ray was not obtained or if there was no evidence of pneumonia.

Box 6-1. Inclusion and exclusion criteria for cases and controls

Inclusion criteria for cases

- Age 18 years or over
- Resident in Blantyre city
- Reported cough **OR** chest pain **OR** breathlessness **OR** hemoptysis
- Reported fever **OR** recorded fever (≥38°C)
- Crepitations **OR** pleural rub **OR** bronchial breathing
- Radiological changes judged to be new and consistent with pneumonia, without another obvious cause
- Requires hospitalisation

Exclusion criteria for cases

- Pre–admission diagnosis of terminal illness (*e.g.*, metastatic malignancy, terminal AIDS)
- Current anti–tuberculosis treatment or evidence of current tuberculosis infection
- Prior hospitalisation within the last 4 weeks
- Prior participation in the study
- Lives in a residential institution (*e.g.*, prison)
- Death prior to follow–up assessment
- Alternative diagnosis explaining their presentation
- Symptoms for 14 days or more

Inclusion criteria for controls

- Age 18 years or over
- Resident in Blantyre city

Exclusion criteria for controls

- Diagnosis of terminal illness (*e.g.*, metastatic malignancy, terminal AIDS)
- Current anti–tuberculosis treatment or evidence of current tuberculosis infection
- Hospitalisation for a pneumonia–like illness in the past 4 months or current pneumonia–like illness
- Current symptoms of pneumonia
- Prior participation in the study
- Lives in a residential institution (*e.g.*, prison)
- Death prior to follow–up assessment
- Utilizes private health care facilities if has illness requiring hospitalisation

6.2.2.2. Controls

Controls, recruited from the community, were defined by absence of pneumonia: willing volunteers were screened for inclusion and exclusion criteria (Box 6-1).

Recruitment was stratified by HIV status to enable the data to be analysed as two separate case–control studies. Within these two sub-groups, controls were frequency–matched to cases by age (18–34 years or ≥35 years) and sex. As poverty was a risk factor of interest (and is inextricably linked to household air pollution exposure), no matching on socioeconomic status was undertaken but controls were restricted to those who used government (not private) healthcare facilities for non-routine treatment. Controls (and cases) were restricted to individuals who lived within the city of Blantyre, and as cases resided in neighbourhoods spread all across the city, controls were recruited from randomly selected neighbourhoods across the city (as described below) to ensure good geographical and socioeconomic variation. To ensure the absence of pneumonia in controls, the study team were trained to enquire about acute respiratory symptoms (as per the exclusion criteria), and in the event of uncertainty, this was discussed with a study doctor.

To identify potential controls, residential census enumeration areas were randomly selected from all enumeration areas within Blantyre city, with selection weighted by population size. Field workers followed randomly generated routes within the enumeration areas and screened all potential participants in each household along the route. A maximum of one individual was recruited per household, selected randomly. Screening continued until 2 controls had been recruited from that enumeration area.

As more HIV-positive than HIV-negative controls were needed (see section 6.2.5.), but the prevalence of HIV in Blantyre is approximately 18%, all potential controls underwent a HIV test as part of their screening process (unless their known HIV status could be confirmed). Recruitment targets were set for each 3-month period to ensure that the correct ratios of individuals were recruited according to their HIV status, age and gender.

To supplement door–to–door recruitment, HIV–positive individuals attending community antiretroviral clinics within Blantyre city were also screened.

Chest x-rays were not performed in controls as, due to the potential of harm of radiation, it was deemed that the absence of symptoms was sufficient to exclude the presence of pneumonia.

6.2.3. Study procedures

6.2.3.1. In-patient case assessment

Initial assessment of cases included medical history and examination by study clinical officers, and diagnostic tests (Box 6-2). Pneumonia was confirmed on review of a chest X-ray by a study doctor.

6.2.3.2. Follow-up assessments

Follow-up assessments were conducted in the participants' homes. For cases, assessments were conducted a minimum of 2 months after their admission for pneumonia to allow time for recovery to normal function, but within a period of 4 months. For controls, the assessments were usually conducted within a week of recruitment.

Box 6-2. Hospital diagnostic tests for pneumonia cases

- HIV test +/- CD4 count
- Malaria rapid diagnostic test
- Blood culture
- BinaxNOW® *Streptococcus pneumoniae* urinary antigen
- Sputum for acid-fast bacilli smear, mycobacterial culture, and GeneXpert® MTB/RIF assay
- Pleural fluid specimen for acid-fast bacilli smear and mycobacterial culture (if clinically indicated)
- Chest X-ray

Air pollution exposure monitoring

Continuous ambulatory and household monitoring of $PM_{2.5}$ (µg/m^3) and CO (ppm) was performed for 48 hours. Participants wore backpacks with Aprovecho Indoor Air Pollution meters ($PM_{2.5}$ and CO) for ambulatory monitoring. UCB-PATS ($PM_{2.5}$) and Lascar EL-USB-CO Data Logger monitors (CO) were placed 1 meter (m) from the household's cooking stove or fire at an elevation of 1 m.

Spirometry

Height and weight were measured in a standardized manner to allow calculation of predicted spirometry values. Spirometry was conducted using an EasyOne Spirometer (ndd Medical Technologies, Zurich, Switzerland) to ATS standards (275). The Third National Health and Nutrition Examination Survey

(NHANES III) reference ranges, corrected for Caucasian and African-American populations, were used to calculate predicted values. Results calculated using Caucasian reference ranges were used in the primary analysis (to allow comparison to the findings of BOLD studies (121)), and secondary analyses were performed using African-American reference range values. Two reviewers independently performed quality assurance and interpreted the spirometry data.

Questionnaires

Questionnaires, including items from the Burden of Obstructive Lung Disease (BOLD) questionnaires (284), were used to evaluate a range of potential risk factors and socioeconomic status. The questions taken from the BOLD questionnaires used the Chichewa translations that had been approved by the BOLD Study Centre and used in the previous BOLD study in Malawi. All other questions were translated to Chichewa by translation staff at MLW Clinical Research Programme, and checked for accuracy by study team members whose mother tongue is Chichewa.

Tuberculosis diagnostics

Cases who had not submitted sputum samples during their in-patient admission and controls who reported chronic cough during their follow up assessment were invited to attend the hospital for sputum induction (conducted as per the methods in Chapter 4) in order to perform tuberculosis diagnostics as above.

6.2.4. Exposures of interest

6.2.4.1. Primary exposures of interest

The primary exposures of interest were mean ambulatory $PM_{2.5}$ exposure and presence of CRD (defined using a composite questionnaire assessment (Box 6-3)).

The definition for CRD used was deliberately inclusive of a range of potential chronic respiratory problems, and did not constrain the variable to recognised western diagnoses. This approach was chosen as - due to multiple respiratory insults experienced by individuals throughout their life - the spectrum CRD seen in sub-Saharan is different to that seen the UK (41). A pragmatic approach was taken by using a predominantly symptom based definition, as individuals who are experiencing symptoms are more likely have clinically significant disease than those who don't.

Box 6-3. Composite definition of chronic respiratory disease

Answering affirmative to any of the following in the BOLD questionnaire:

- Current usual cough
- Current usual sputum production
- Current breathlessness
- Wheeze in past 12 months
- Ever had diagnosis of emphysema
- Current diagnosis of chronic bronchitis
- Current diagnosis of asthma
- Current long-term respiratory medication

Note: Cases were asked to recall their status from 6 months previously, prior to their episode of pneumonia.

6.2.4.2. Secondary exposures of interest

The following exposures were also evaluated as potential risk factors for pneumonia:

- Mean ambulatory CO exposure
- Mean household CO exposure
- Mean household $PM_{2.5}$ exposure
- History of previous respiratory disease or symptoms
- Spirometric evidence of airway obstruction or restriction
- Self-reported air pollution exposures (including household, outdoor, occupational)
- Self-reported tobacco smoke exposure (current and previous smoking and passive exposures, from cigarettes, cigars and other forms of tobacco smoke inhalation)
- CD4 count (HIV-positive only)
- Antiretroviral use (HIV-positive only)
- Body mass index (BMI)
- Alcohol use
- Socio-economic status (using poverty indicators including asset ownership, education, housing conditions)

- Contact with children, people with illnesses and animals
- Marital status

6.2.5. Sample size

Based on assumptions of α=0.05, β=0.2, and an estimated percentage of controls with CRD of at least 15%, the target sample size was 160 cases and 160 controls in the HIV-positive sub-group (ratio 1:1) to detect an OR of 2.2 or greater. A smaller exploratory study was planned with 60 cases and 90 controls in the HIV-negative sub-group (ratio 1:1.5).

6.2.5.1. Recruitment targets

Based on preliminary data from the MARISO study, it was anticipated that 30% of patients (who will be predominantly recruited prior to a chest x-ray being taken) would subsequently be found to be ineligible (as only 88% of patients would get an x-ray, and only 80% of these will have x-ray confirmed pneumonia). It was estimated that 30% of HIV-positive cases and 10% of HIV-negative cases would have died or started tuberculosis treatment by 2-4 month follow-up, and 20% of the remaining participants will be lost to follow up. Therefore case recruitment targets were set at HIV 406 positive cases and 118 HIV-negative cases.

10% of controls were expected to be lost to follow up, therefore recruitment targets were 178 HIV-positive controls and 100 HIV-negative controls. Based on an 18% HIV-positive rate in the community, it was anticipated that approximately 1000 individuals would undergo HIV testing to find 178 HIV-positive controls.

6.2.6. Statistical considerations

Data files were exported to Stata 13.1 (Statacorp, College Station, Texas, USA) for analysis. Missing air pollution exposure data were imputed using spatial interpolation. Missing questionnaire data were imputed using multivariate multinomial models by exploiting their association with other observed variables. A socioeconomic status score was generated using principal components analysis, based on data regarding asset-based measures, education level, and household characteristics (the variables used are indicated in Table 6-4) (285).

Comparisons between clinical and exposure data were made using Independent t-tests, Mann-Whitney U tests and Pearson's Chi-squared tests. Bivariate associations between various outcomes and exposures were made using logistic regression, one-way ANOVA and Pearson's Chi^2 tests and

Spearman's rank correlation tests. A p value of <0.05 was considered statistically significant. No adjustments for multiple testing were made.

Table 6-1: *A priori* potential confounders included in the multivariate logistic regression model.

Forced variables included in the logistic regression model	*A priori* potential confounder with Likelihood Test Ratio *P* value <0.2 therefore entered into the logistic regression model	*A priori* potential confounder with Likelihood Test Ratio *P* value >0.2 therefore not entered into the logistic regression model
Age	Alcohol intake	Socioeconomic status quintile
Gender	Smoking status (all forms)	Mean household $PM_{2.5}$ exposure*
	BMI	Primary cooking fuel
	CD4 count	Ventilation whilst cooking
	Antiretroviral therapy	Pollution from heating/lighting
	Co-trimoxazole prophylaxis	Education level
	Chronic respiratory disease	Passive tobacco smoke exposure
	Mean ambulatory $PM_{2.5}$ exposure*	Roofing material
	Mean ambulatory CO exposure*	Floor material
	Mean household CO exposure*	Number of people per room in household
	Cooking with solid fuel frequency	Contact with children
	Civil status	Population density
	Current occupation	
	Carer for somebody with chronic illness	
	Wall material	
	Animal ownership	
	Work related dust/smoke exposures	

*$PM_{2.5}$ and CO exposures included as potential confounders for CRD analysis only.

BMI: body mass index; $PM_{2.5}$: particulate matter with <2.5µm diameter; CO: carbon monoxide.

For analysis of the HIV-positive sub-group, univariate logistic including *a priori* potential confounders was performed (a list of *a priori* potential confounders can be found in Table 6-1). Mechanistically plausible potential confounders with a univariate likelihood ratio test p-value of <0.2 were entered into

a multivariate forward stepwise logistic regression model (criteria for entry $p<0.05$ and removal $p>0.1$) for each of the main exposures of interest. For analysis of the HIV-negative sub-group, owing to the smaller sample size, multivariate logistic regression was performed, with only matching factors (age and gender) entered into the model as forced variables. To test the hypothesis that pneumonia cases are spatially clustered, we used generalized additive models and smoothing latitude and longitude over the geographic reach of the study area (286).

6.2.6.1. Interim analysis

An interim analysis was performed in August 2015 after approximately 50% of the planned sample size had been recruited. Although an interim analysis had not been pre-specified, this was undertaken to inform a decision about whether to extend recruitment beyond the planned recruitment period, as recruitment had been slower than anticipated. Bootstrapping was used to create a simulated dataset (using random sampling with replacement based on the existing data already collected), which was combined with the existing dataset, to create a synthetic complete dataset. This was repeated 50 times (to create 50 synthetic complete datasets) in order to explore the probability of obtaining a significant result, based on the assumption that future data will be similar to that already collected. Point estimates and their standard errors were calculated using logistic regression.

6.2.7. Ethical considerations

This study was approved by the College of Medicine REC, University of Malawi (P.02/14/1518) and the LSTM REC (14.016). All participants gave written informed consent prior to participation in the study.

6.3. Results

6.3.1. Participant recruitment

The interim analysis indicated that there was a high chance of detecting (with at least 80% power) a significant association between ambulatory mean CO exposure (but not ambulatory mean $PM_{2.5}$ exposure) and pneumonia, as well as CRD and pneumonia, in the HIV-positive sub-group if recruitment was stopped at the end of the planned recruitment period with a smaller sample size than initially planned (110 cases and 140 controls in the HIV-positive sub-group). Recruitment was therefore stopped in February 2016, as originally planned.

We screened 2148 and 1492 potential cases and controls, respectively, between July 2014 and February 2016 (Figure 6-1). Of the screened cases, 58.5% were men with a median age of 36 years (IQR 30-47). Of

the screened controls, 61.6% were men, with a median age of 30 (IQR 23-40). Of the 1273 potential controls identified via door-to-door screening, 540 potential controls (42.4%) underwent HIV testing as part of their screening process (41 HIV-positive, 496 HIV-negative, 3 withheld result), 107 (8.4%) already knew their HIV status (76 HIV-positive and 31 HIV-negative), 36 (2.8%) declined to be screened and the remaining 590 (46.4%) were either unavailable for testing or did not reach that part of the screening process due to ineligibility on other criteria. All 219 potential controls screened at health centre HIV clinics were already known to be HIV-positive. From HIV-positive and HIV-negative sub-groups, respectively, we recruited 349 and 79 cases, and 208 and 92 controls (Figure 6-1). Of the recruited participants, 64.7% and 59.3% were male, with a median age of 35 (IQR 30-42, range 18-89) and 35 (IQR 29–43, range 18–78) in the cases and controls, respectively, and lived across Blantyre city (Figure 6-2). The reasons for ineligibility amongst potential cases and controls are listed in Table 6-2.

One hundred and forty-five (117 HIV-positive, 28 HIV-negative) cases and 253 (169 HIV-positive, 84 HIV-negative) controls completed follow-up. Reasons for not completing follow-up amongst recruited cases were subsequent exclusion for ineligibility (265, 61.9%; including lack of radiological evidence of pneumonia [108, 25.2%], commencement of tuberculosis treatment [140, 32.7%], and death [64, 15.0%]), and loss to follow-up (18, 4.2%; Figure 6-1). Twenty-five of the individuals who were started on tuberculosis treatment subsequently died. Among recruited controls, 34 individuals (11.3%) were lost to follow-up and 13 (4.3%) were ineligible and subsequently excluded.

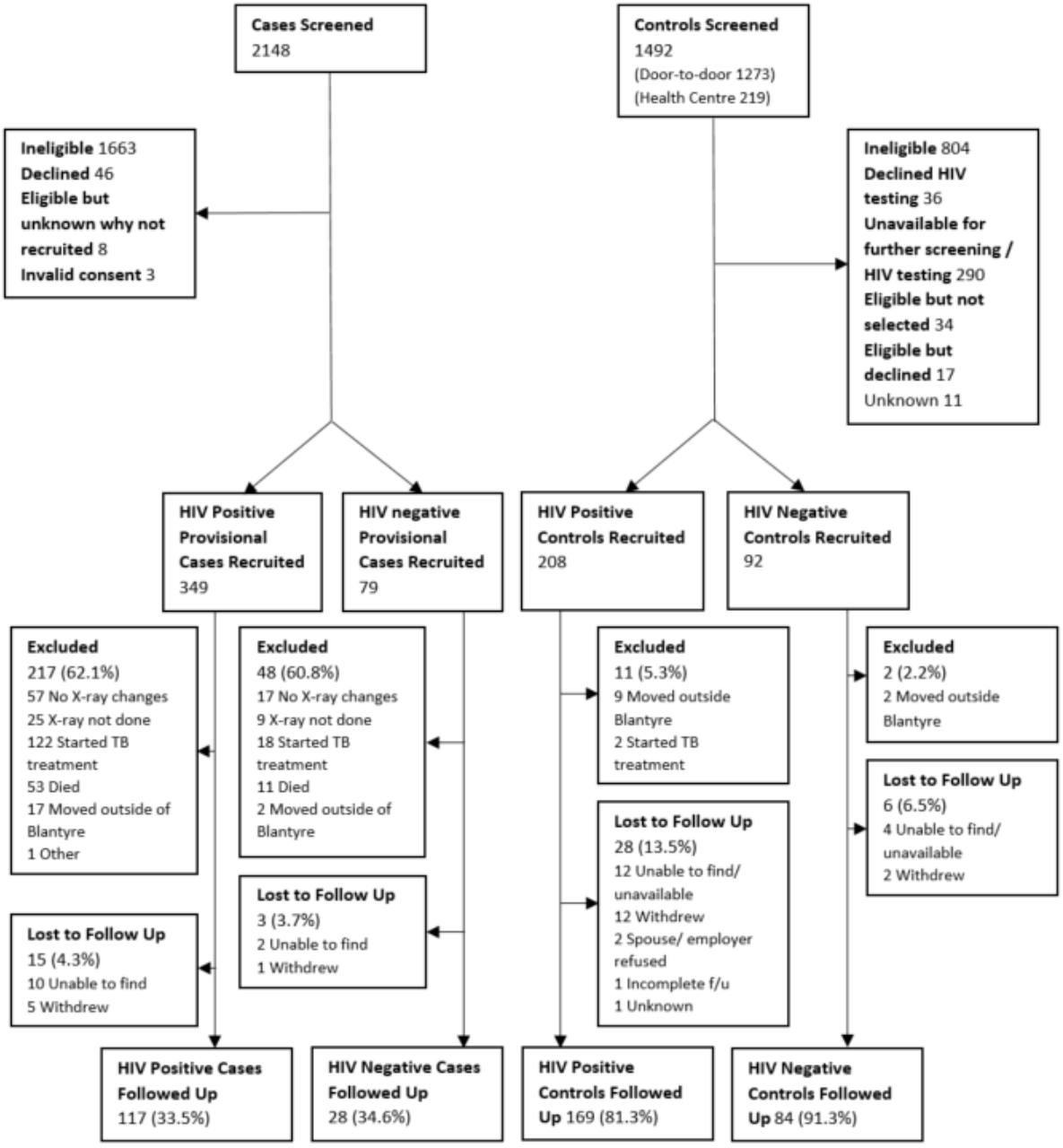

Figure 6-1: Participant flow chart showing the number of cases and controls screened, recruited, and followed up in the HIV–positive and HIV–negative sub-groups.
HIV: Human Immunodeficiency Virus; TB: tuberculosis.

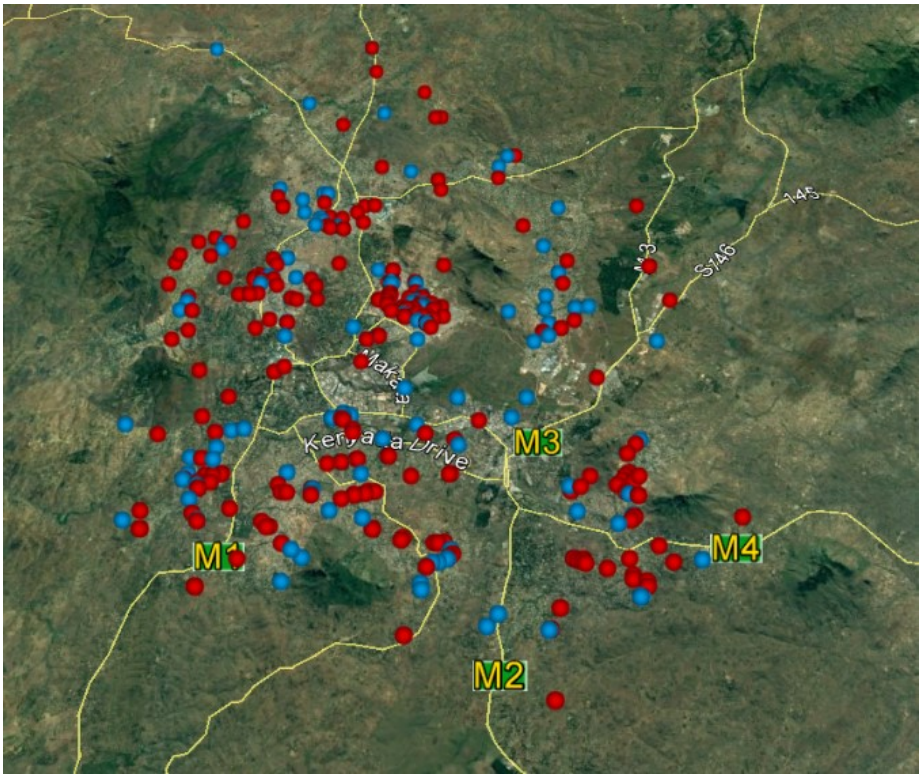

Figure 6-2: Participant household locations.
A map of Blantyre city, Southern Region, Malawi showing the location of case (•) and control (•) households. Mapping software: Google Earth Pro (7.1.5.1157).

6.3.2. Hospital admission details for cases

Details regarding the presentation and in-patient hospital stay for all 428 recruited cases (including those who were subsequently excluded) can be found in Table 6-3. Treatment was sought prior to attending QECH by 276 (64.5%) of all recruited cases. Of those, 221 (80.1%) had attended a primary health care centre and 33 (12.0%), 9 (3.3%) and 5 (1.8%) had attended a private clinic, other hospital and pharmacy, respectively. None reported visiting a traditional healer. Anti-malarial treatment had been taken by 50 (11.7%) cases in the two weeks prior to admission and 16 (3.7%) had taken traditional medicine. Only one person had travelled more than three hours to reach QECH, but 55.84% of recruited cases had travelled for more than one hour.

The symptoms reported by all recruited cases (including those who were subsequently excluded) are shown in Figure 6-3. HIV-positive cases were more likely to report weight loss, but otherwise the symptom profiles were similar between the two groups. Respiratory crepitations were heard on auscultation in 342 (79.9%) and 168 (39.3%), 75 (17.5%), 40 (9.4%) and 17 (4.0%) had bronchial

breathing, signs of a pleural effusion, pleural rub and wheeze, respectively. Abnormalities were detected on examination of the upper respiratory tract in 34 (7.9%) of recruited cases.

Table 6-2: Reasons for ineligibility for potential cases and controls.

Reason for ineligibility	Cases, n (%) (Total screened = 2148; total ineligible = 1663)	Controls, n (%) (Total screened = 1492; total ineligible = 804)
Symptom duration greater than 14 days	913 (42.5)	N/A
Didn't meet age/gender/HIV criteria for stratified recruitment	N/A	676 (45.3)
Lack of clinical signs consistent with pneumonia	416 (19.4)	N/A
Living outside of urban Blantyre	385 (17.9)	-
Absence of fever	304 (14.2)	N/A
Hospitalisation within past 4 weeks	134 (6.2)	-
Obvious alternative diagnosis	131 (6.1)	N/A
No x-ray taken or no changes present	108 (5.0)	N/A
Current tuberculosis treatment	95 (4.4)	55 (3.7)
Recent pneumonia-like illness	N/A	45 (3.0)
Doesn't use government healthcare facilities	N/A	21 (1.4)
Terminal illness	38 (1.8)	11 (0.7)
Lack of relevant symptoms	37 (1.7)	N/A
Age <18 years	31 (1.4)	-
Didn't require hospitalisation	14 (0.7)	N/A
Lives in an institution	14 (0.7)	-
Previously enrolled in AIR Study	12 (0.6)	-
Unknown	72 (3.4)	-

N/A = not applicable (eligibility criteria not applicable to that study group).

HIV: Human Immunodeficiency Virus; AIR: Acute Infection of the Respiratory tract Study.

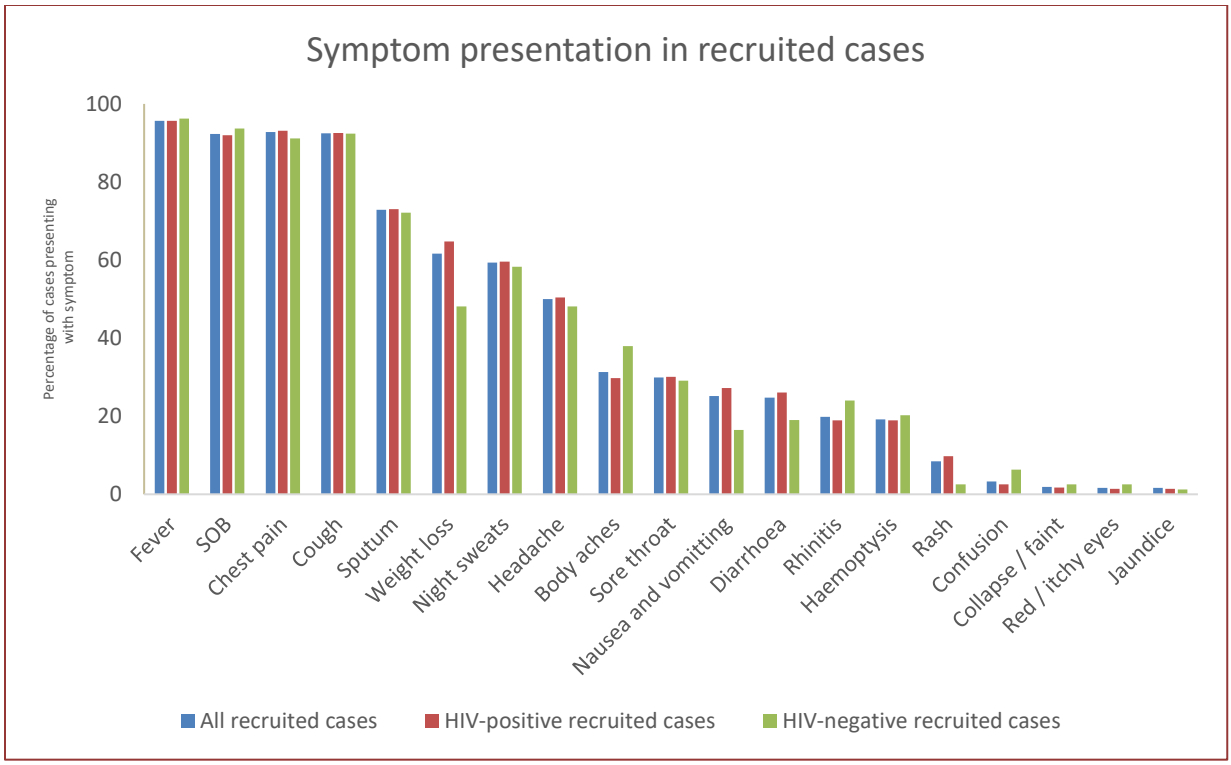

Figure 6-3: Symptoms at presentation for all recruited cases
SOB: shortness of breath; HIV: Human Immunodeficiency Virus.

Blood culture results were available for 400 (93.4%) of all recruited cases. Of these, 27 (6.8%) were positive (11 (40.7%) *Salmonella* species, 5 (18.5%) *Streptococcus pneumoniae*, 3 (11.1%) *Escherichia* coli, 2 (7.41%) *Staphylococcus aureus* and 2 (7.41%) *Cryptococcus neoformans*. BinaxNOW *Streptococcus pneumoniae* urinary antigen results were available for 386 (90.2%) recruited cases. Of the 82 (21.2%) with a positive urinary antigen result, 8 (9.8%) also had a positive blood culture result (although only 2 of these cultured *Streptococcus pneumoniae*). Three individuals with *Streptococcus pneumoniae* identified in their blood culture had a negative BinaxNOW *Streptococcus pneumoniae* urinary antigen result.

At least one form of tuberculosis diagnostic result was available for 323 (75.5%) of recruited cases. Of these 300 (92.88),282 (87.3%), 266 (82.4%), 13 (4.0%) and 12 (3.7%) had a sputum GeneXpert® result, sputum smear, sputum culture, pleural fluid culture and pleural fluid smear result available, respectively. Of these 323 individuals, 67 (20.7%) had a positive *Mycobacterium tuberculosis* result and 8 (2.48%) had a non-tuberculous *Mycobacterium* identified (and were therefore excluded as cases from the AIR Study). A fifth (94, 21.9%) of all recruited cases were commenced on tuberculosis treatment during their hospital admission (and were therefore excluded as cases from the AIR Study). A further 46

(10.7%) of participants were started on tuberculosis treatment following discharge but prior to their follow-up appointment. Seven (1.64%) of the recruited cases were known to be started on treatment for *Pneumocystis jiroveci* pneumonia during their hospital admission, 3 of whom died as an in-patient.

Overall, 349 recruited cases (81.5%) were HIV-positive, with 33 (9.5%) of those being newly diagnosed. One hundred and eighty-eight (78.0%) of those previously known to be HIV-positive were taking antiretroviral treatment prior to admission. Forty-six (10.7%) patients died prior to hospital discharge and a further 18 (5.2%) died following discharge, prior to follow-up. There was no significant difference in in-patient mortality rates between HIV-positive (40, 11.5%) and HIV-negative (6, 7.6%) patients (Pearson's chi-square test = 0.92, p=0.337).

The majority of patients (394 (92.1%)) had a chest x-ray taken; 11 (34.4%) of those without a chest x-ray died. Of those with a chest x-ray, 320 (81.2%) had changes considered to be consistent with pneumonia. Despite a case definition mandating a symptom duration of 14 days or less, 53 (16.6%) of the 320 recruited cases with chest x-ray changes had a confirmed diagnosis of tuberculosis (20.7% of those with diagnostic results available) and a further 60 (18.8%) were commenced on tuberculosis treatment prior to their follow up appointment on the basis of clinical suspicion. The 207 remaining patients (48.4% of all recruited cases) were treated as community acquired pneumonia.

6.3.2.1. Characteristics of confirmed pneumonia cases

Of the 207 (48.4%) considered to have pneumonia, 126 (60.9%) were male and there was a median age of 36 (IQR 30-45)). HIV prevalence was 81.6% (169 HIV-positive individuals). Median baseline CD4 count amongst this group was 109.5 cells/µl (IQR 43-207) and 67 (39.6%) were not on ART. Of the patients treated as pneumonia, 50 (24.2%) had confirmed pneumococcal pneumonia (47 positive BinaxNOW *Streptococcus pneumoniae* urinary antigen results, 4 positive blood cultures). Overall mortality in this group was 14.0% (15 (7.25%) died as an in-patient and a further 14 (6.8%) subsequently died before follow up).

6.3.2.2. Comparison of cases who completed follow up and those who did not

Comparisons between cases who completed follow-up (confirmed pneumonia) and those who did not (predominantly due to ineligibility (see Figure 6-1): a mixture of individuals with non-pneumonia respiratory infections, tuberculosis and pneumonia) were made to explore evidence of potential selection bias (Table 6-3). Vital signs were broadly similar between the two groups. Cases who completed follow-up were older (mean age 36 years vs 35 years, p = 0.040), and had a statistically significant shorter duration of symptoms prior to admission (although median symptom duration 7 days in both groups), and shorter length of admission (5 days vs 7 days, p = 0.021) compared to those who did not complete follow up. Those who completed study follow-up and were HIV-positive were less likely to have an existing diagnosis of HIV (64.9% vs 71.1%, p = 0.023) and had a higher admission CD4 count than those who were not followed up (median 119 cells/µl [IQR 47–205] vs. median 86 cells/µl [IQR 30–185], p = 0.029).

6.3.3. Characteristics of cases and controls

Details of demographics, socioeconomic status, household characteristics and health status for cases and controls who completed follow up can be found in Table 6-4. Out of all participants, only one (a HIV-positive case) reported they had received pneumococcal and *Haemophilus influenza* vaccinations, and none had received an influenza vaccination.

In the HIV-positive sub-group, cases and controls had similar ages and were predominantly male (58.1% and 55.0%, respectively). Just over half of all HIV-positive cases and controls had never alcohol (53.9% and 55.6%, respectively), but cases were more likely to be previous drinkers than controls (39.3% vs 23.7%) and less likely to be current drinkers than controls (6.8% vs 20.7%). Current smoking was more common amongst HIV-positive cases than controls (3.4% vs 11.2%). There was no consistent difference in measures of socioeconomic status between HIV-positive cases and controls, and their households had similar characteristics. BMI was lower in HIV-positive cases than controls (mean 19.9 kg/m^2 (STD 2.5 kg/m^2) vs 21 kg/m^2 (STD 3.9 kg/m^2). CD4 count was lower in cases than controls (median 129 cells/µl (IQR 49-209 cells/µl) vs 355 cells/µl (IQR 236-492 cells/µl), and cases were less likely to be taking antiretroviral therapy or co-trimoxazole than controls (58.1% vs 79.9% and 57.3% and 75.2%, respectively). Very few individuals had previously been told they have non-respiratory co-morbidities.

Table 6-3: Baseline hospital data for all recruited cases.

	Cases who completed follow-up (total n=145)	Cases who did not complete follow-up (total n=283)	*P* value
Male, n (%)	91 (62.8)	186 (65.7)	0.543**
Age (years), median [IQR]	36 [31-45]	35 [30-41]	0.040††
Symptom duration (days), median [IQR] *	7 [5–8]	7 [5–10]	0.007††
Length of admission (days), median [IQR] *	5 [4–8]	7 [4–12]	0.021††
Hospital outcome, n (%)			<0.001**
Alive	145 (100.0)	232 (82.0)	
Dead	0 (0)	46 (16.3)	
Unknown	0 (0)	5 (1.8)	
Pre-hospital antibiotics, n (%)			0.742**
Yes	79 (54.5)	158 (55.8)	
No	59 (40.7)	111 (39.2)	
Unknown	7 (4.8)	14 (4.9)	
Systolic blood pressure (mmHg), mean [STD] *	104.4 [22.5]	112.1 [79.5]	0.263‡‡
Diastolic blood pressure (mmHg), mean [STD] *	67.0 [14.5]	75.5 [81.1]	0.223‡‡
Heart rate (bpm), mean [STD] *	116.5 [20.0]	116.5 [22.6]	0.995‡‡
Respiratory rate (bpm), median [IQR] *	28 [23–36]	28 [24–34]	0.748††
Oxygen saturation (%), median [IQR] *	95 [91–97]	95 [90–98]	0.344††
Temperature (°C), median [IQR]	38.2 [37.1–39.0]	37.8 [36.7–38.7]	0.023††
HIV-positive, n (%)	117 (80.7)	232 (82.0)	0.745**
Diagnosis of HIV, n (%)†			0.071**
Previously known	76 (64.9)	165 (71.1)	
New diagnosis	17 (14.5)	16 (6.9)	
Unknown	24 (20.5)	51 (22.0)	
CD4 (cells/µl), median [IQR] †*	119 [47-205]	86 [30-185]	0.029††
Pre-hospital antiretroviral treatment, n (%)‡	60 (79.0)	128 (77.6)	0.767**
Pre-hospital co-trimoxazole prophylaxis, n (%)‡	59 (77.6)	120 (72.7)	0.418**

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Table 6-3 continued	Cases who completed follow-up (total n=145)	Cases who did not complete follow-up (total n=283)	*P* value
Chest X-ray changes consistent with pneumonia, n (%)*	145 (100)	190 (73.4)	<0.001**
Confirmed diagnosis of tuberculosis, n (%)*	0 (0)	67 (33.0)	<0.001**
In-hospital commencement of tuberculosis treatment, n (%)*	0 (0)	94 (33.7)	<0.001**
Positive malaria rapid diagnostic test, n (%)*	4 (3.0)	5 (1.9)	0.511**
Positive blood culture, n (%)*	7 (5.3)	20 (7.5)	0.403**
Positive BinaxNOW *Streptococcus pneumoniae* urinary antigen, n (%)*	34 (25.2)	48 (19.1)	0.165**

*Missing data was not imputed; †of those who are HIV-positive; ‡of those who were previously known to be HIV-positive; ** compared using Pearson's Chi-squared test; ††compared using Mann-Whitney U tests; ‡‡compared using Independent t-tests.

IQR: interquartile range; mmHg: millimetres of mercury; STD: standard deviation; bpm: beats/breaths per minute; HIV: Human Immunodeficiency Virus.

In the HIV-negative sub-group, cases were slightly older than controls (median 39 years (IQR 30-64 years) vs 35 years (IQR 26-42 years), and males were more common amongst cases than controls (82.1 % vs 64.3%). Fewer HIV-negative cases had never drunk alcohol or never smoked than controls (39.3% vs 61.9% and 26.4% vs 81%, respectively). Cases were more commonly in the lowest socioeconomic status quintile than controls (35.7 vs 13.1%), and they had poorer quality roofing and flooring in their homes. Similarly to the HIV-positive sub-group, cases in the HIV-negative sub-group had a lower BMI than controls, but average values were higher in the HIV-negative sub-groups compared to the HIV-positive sub-groups.

Table 6-4: Participant characteristics for HIV-positive and HIV-negative cases and controls, and univariate analysis of the association between exposures and pneumonia.

Exposures	HIV-positive sub-group				HIV-negative sub-group			
	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Participant characteristics								
Age (years), median [IQR]	36 [31-43]	36 [32-44]	--	--	39 [30-64]	35 [26-42]	--	--
Gender, n (%)			--	--			--	--
Male	68 (58.1)	93 (55.0)			23 (82.1)	54 (64.3)		
Female	49 (41.9)	76 (45.0)			5 (17.9)	30 (35.7)		
Civil status, n (%)								
Married	68 (58.1)	121 (71.6)	1	--	20 (71.4)	54 (64.3)	1	--
Widowed	11 (9.4)	18 (10.7)	1.09 (0.49-2.44)	0.839	2 (7.1)	4 (4.8)	1.35 (0.23-7.95)	0.740
Divorced	14 (12.0)	11 (6.5)	2.26 (0.97-5.26)	0.058	3 (10.7)	2 (2.4)	4.05 (0.63-26.05)	0.141
Separated	7 (6.0)	2 (1.2)	6.23 (1.25-30.83)	0.025	0 (0)	3 (3.6)	1	--
Single	17 (14.5)	17 (10.1)	1.78 (0.85-3.71)	0.124	3 (10.7	21 (25.0)	0.39 (0.22-0.62)	<0.001
Smoking status (all forms), n (%)								
Never smoked	85 (72.7)	123 (72.8)	1	--	13 (26.4)	68 (81.0)	1	--
Ex-smoker	28 (23.9)	27 (16.0)	1.50 (0.83-2.73)	0.182	10 (35.7)	7 (8.3)	7.47 (2.41-12.2)	0.001
Current smoker	4 (3.4)	19 (11.2)	0.30 (0.10-0.93)	0.036	5 (17.9)	9 (10.7)	2.9 1 (0.84-10.08)	0.093
Passive tobacco smoke exposure, n (%)								
No	65 (55.6)	90 (53.3)	1	--	13 (46.4)	54 (64.3)	1	--
Yes	42 (44.4)	79 (46.8)	0.91 (0.57-1.47)	0.701	15 (53.6)	30 (35.7)	2.08 (0.87-4.94)	0.098

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Table 6-4 continued	HIV-positive sub-group				HIV-negative sub-group			
Exposures	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Alcohol intake, n (%)								
Never	63 (53.9)	94 (55.6)	1	--	11 (39.3)	52 (61.9)	1	--
Previous drinker	46 (39.3)	40 (23.7)	1.72 (1.01-2.92)	0.046	13 (46.4)	15 (17.9)	4.10 (1.53-11.00)	0.005
Current drinker	8 (6.8)	35 (20.7)	0.34 (0.15-0.78)	0.011	4 (14.3)	17 (20.2)	1.11 (0.31-3.96)	0.869
Carer for person with chronic illness, n (%)								
No	77 (65.8)	97 (57.4)	1	--	22 (78.6)	58 (69.1)	1	--
Yes	40 (34.2)	72 (42.6)	0.70 (0.43-1.14)	0.137	6 (21.4)	26 (31.0)	0.61 (0.22-1.68)	0.337
Socioeconomic factors								
Socioeconomic status quintile, n (%)								
Highest	19 (16.2)	26 (15.4)	1	--	6 (21.4)	28 (33.3)	1	--
High	25 (21.4)	40 (23.7)	0.86 (0.39-1.86)	0.692	2 (7.1)	14 (16.7)	0.67 (0.12-3.74)	0.645
Middle	26 (22.2)	33 (19.5)	1.08 (0.49-2.36)	0.851	7 (25.0)	15 (17.9)	2.18 (0.62-7.66)	0.225
Low	22 (18.8)	37 (21.9)	0.81 (0.37-1.80)	0.610	3 (10.7)	16 (19.1)	0.86 (0.19-3.98)	0.863
Lowest	25 (21.4)	33 (19.5)	1.04 (0.48-2.28)	0.929	10 (35.7)	11 (13.1)	4.24 (1.24-14.50)	0.021
Household member has bank account, n (%)[§]								
No	71 (60.7)	99 (58.6)	1	--	20 (71.4)	39 (46.4)	1	--
Yes	46 (39.3)	70 (41.4)	0.92 (0.56-1.48)	0.722	8 (28.6)	45 (53.6)	0.35 (0.14-0.87)	0.025

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Table 6-4 continued	HIV-positive sub-group				HIV-negative sub-group			
Exposures	**Cases (n=117)**	**Controls (n=169)**	**Unadjusted OR (95% CI)**	***P* value**	**Cases (n=28)**	**Controls (n=84)**	**Unadjusted OR (95% CI)**	***P* value**
Education level, n (%)								
Higher education§	7 (6.0)	12 (7.1)	1	--	2 (7.1)	12 (14.3)	1	--
Secondary only§	45 (38.5)	59 (35.1)	1.31 (0.48-3.59)	0.603	4 (14.3)	33 (39.3)	0.73 (0.12-4.50)	0.732
Primary only	59 (50.4)	90 (53.8)	1.12 (0.41-3.02)	0.817	18 (64.3)	33 (39.3)	3.27 (0.66-16.26)	0.147
None	6 (5.1)	7 (4.2)	1.47 (0.35-6.17)	0.599	4 (14.3)	5 (6.0)	4.8 (0.65-35.20)	0.123
Unknown	0 (0)	0 (0)	--	--	0 (0)	1 (1.2)	--	--
Number of school years completed, median [IQR]*	8 [5-12]	8 [6-11]	--	--	6 [3-8]	10 [7-12]	--	--
Current occupation, n (%)								
Paid employee	40 (34.2)	47 (27.8)	1	--	12 (42.9)	29 (34.5)	1	--
Paid domestic worker	2 (1.7)	2 (1.2)	1.18 (0.16-8.72)	0.875	0 (0)	3 (3.6)	1	--
Self-employed	40 (34.2)	37 (21.9)	1.27 (0.69-2.35)	0.445	7 (25.0)	16 (19.1)	1.06 (0.27-1.98)	0.922
Unemployed	32 (27.4)	78 (46.2)	0.48 (0.27-0.87)	0.015	9 (32.1)	30 (35.7)	0.73 (0.27-1.98)	0.530
Unpaid family worker	1 (0.9)	0 (0)	1	--	0 (0)	0 (0)	--	--
Student	2 (1.7)	5 (3.0)	0.47 (0.09-2.56)	0.382	0 (0)	6 (7.1)	1	--
Not enough money for soap in past year, n (%)§								
No	95 (81.2)	131 (77.5)	1	--	20 (71.4)	68 (80.1)	1	--
Yes	22 (18.8)	38 (22.5)	0.80 (0.44-1.44)	0.453	8 (28.6)	16 (19.9)	1.7 (0.64-4.55)	0.291

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Table 6-4 continued	HIV-positive sub-group				HIV-negative sub-group			
Exposures	**Cases (n=117)**	**Controls (n=169)**	**Unadjusted OR (95% CI)**	***P* value**	**Cases (n=28)**	**Controls (n=84)**	**Unadjusted OR (95% CI)**	***P* value**
Household ownership of assets, n (%)			--	--			--	--
Electricity§	61 (52.1)	76 (45.0)			11 (39.3)	49 (58.3)		
Flush toilet§	2 (1.7)	9 (5.3)			3 (10.7)	11 (13.1)		
Television§	48 (41.0)	62 (36.7)			10 (35.7)	46 (54.8)		
Telephone	0	3 (1.8)			1 (3.6)	2 (2.4)		
Mobile telephone§	98 (83.8)	144 (85.2)			23 (82.1)	72 (85.7)		
Radio§	83 (70.9)	116 (68.6)			23 (82.1)	67 (79.8)		
Refrigerator§	21 (18.0)	32 (18.9)			6 (21.4)	25 (29.8)		
Bed with mattress§	85 (72.7)	132 (78.1)			17 (60.7)	70 (83.3)		
Car	2 (1.7)	7 (4.1)			1 (3.6)	2 (2.41)		
Motorbike/moped	2 (1.7)	0			1 (3.6)	1 (1.2)		
Washing machine	0	0			0	0		
Home	71 (61.2)	109 (64.9)			14 (50.0)	38 (45.8)		
Indoor bath or shower§	5 (4.3)	10 (5.9)			3 (10.7)	12 (14.3)		
Indoor tap§	4 (3.4)	10 (5.9)			3 (10.7)	11 (13.1)		
Outdoor tap§	19 (16.2)	35 (20.7)			5 (17.9)	16 (19.1)		
Mosquito net§	89 (77.4)	138 (81.7)			19 (67.9)	71 (85.5)		
Animal ownership, n (%)§								
No animals	86 (73.5)	111 (65.7)	1	--	13 (46.4)	59 (70.2)	1	--
Owns animals	31 (26.5)	58 (34.3)	0.69 (0.41-1.16)	0.161	15 (53.6)	25 (29.8)	2.72 (1.13-6.55)	0.025

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Table 6-4 continued	HIV-positive sub-group				HIV-negative sub-group			
Exposures	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Sleeps in same room as animals, n (%)								
No	117(100.0)	167 (98.8)	1	--	27 (96.4)	81 (96.4)	1	--
Yes	0 (0)	2 (1.2)	1	--	1 (3.6)	3 (3.6)	1.00 (0.10-10.02)	1.000
Household member went hungry for lack of money in past year, n (%)			--	--			--	--
Most days§	2 (1.7)	5 (3.0)			0	2 (2.4)		
Certain times of year	41 (35.3)	62 (36.9)			11 (40.7)	24 (28.6)		
Occasionally	7 (6.0)	9 (5.4)			1 (3.7)	1 (1.2)		
Never	66 (56.9)	92 (54.8)			15 (55.6)	57 (67.9)		
Household member went hungry for lack of money when age 5, n (%)			--	--			--	--
Most days	2 (1.7)	6 (3.6)			1 (3.6)	7 (8.3)		
Certain times of year	51 (43.6)	73 (43.2)			16 (57.1)	33 (39.3)		
Occasionally	10 (8.6)	15 (8.9)			3 (10.7)	1 (1.2)		
Never	54 (46.2)	73 (43.2)			8 (28.6)	43 (51.2)		
Household characteristics								
Roofing material, n (%)§								
Finished	112 (95.7)	164 (97.0)	1	--	25 (89.3)	82 (97.6)	1	--
Rudimentary	0 (0)	0 (0)	--	--	0 (0)	0 (0)	--	--
Natural	5 (4.3)	5 (3.0)	1.46 (0.41-5.18)	0.554	3 (10.7)	2 (2.4)	4.92 (0.78-31.11)	0.090

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Table 6-4 continued	HIV-positive sub-group				HIV-negative sub-group			
Exposures	**Cases (n=117)**	**Controls (n=169)**	**Unadjusted OR (95% CI)**	***P* value**	**Cases (n=28)**	**Controls (n=84)**	**Unadjusted OR (95% CI)**	***P* value**
Wall material, n (%)[§]								
Finished	79 (67.5)	98 (58.0)	1	--	19 (67.9)	57 (67.9)	1	--
Rudimentary	34 (29.1)	71 (42.0)	0.59 (0.35-0.98)	0.043	8 (28.6)	26 (31.0)	0.92 (0.36-2.38)	0.868
Natural	4 (3.4)	0 (0)	1	--	1 (3.6)	1 (1.2)	3.00 (0.18-50.33)	0.445
Floor material, n (%)[§]								
Finished	93 (79.5)	134 (79.3)	1	--	20 (71.4)	72 (85.7)	1	--
Rudimentary	0 (0)	0 (0)	--	--	0 (0)	0 (0)	--	--
Natural	24 (20.5)	35 (20.7)	0.99 (0.55-1.77)	0.968	8 (28.6)	12 (14.3)	2.40 (0.86-6.67)	0.093
Number of people per room in household, median [IQR]	1.5 [1.0-2.0]	1.5 (1.0-2.0)	1.09 (0.79-1.51)[†]	0.604	1.7 [1.0-2.0]	1.5 [1.0-2.0]	1.15 (0.66-1.99)[†]	0.625
Contact with children, n (%)								
Does not live with children	15 (12.8)	27 (16.0)	1	--	2 (7.1)	14 (16.8)	1	--
Lives with children	102 (87.2)	142 (84.0)	1.29 (0.65-2.55)	0.459	26 (92.9)	70 (83.3)	2.60 (0.55-12.23)	0.226
Population density (1000 people/ km^2), median [IQR]	4.2 [2.2-8.0]	4.2 (2.4-9.9)	0.98 (0.92-1.02)[†]	0.256	3.9 [2.2-7.1]	3.9 [2.2-9.9]	0.96 (0.88-1.06)[†]	0.447
Participant health characteristics								
Body mass index (kg/m^2), mean [STD]	19.9 [2.5]	21.6 [3.9]	0.85 (0.78-0.92)[†]	< 0.001	20.9 [3.8]	23.2 [4.9]	0.84 (0.72-0.98)[†]	0.023
CD4 count (cells/µl), median [IQR]	129 [49-209]	355 (236-492)	0.99 (0.99-0.99)[†]	< 0.001	--	--	--	--

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Table 6-4 continued	HIV-positive sub-group				HIV-negative sub-group			
Exposures	**Cases (n=117)**	**Controls (n=169)**	**Unadjusted OR (95% CI)**	***P* value**	**Cases (n=28)**	**Controls (n=84)**	**Unadjusted OR (95% CI)**	***P* value**
ART, n (%)					--	--	--	--
No	49 (41.9)	39 (23.1)	1	--				
Yes	68 (58.1)	130 (76.9)	0.42 (0.25-0.70)	0.001				
Co-trimoxazole prophylaxis, n (%)					--	--	--	--
No	50 (42.7)	42 (24.9)	1	--				
Yes	67 (57.3)	127 (75.2)	0.44 (0.27-0.73)	0.002				
Ever been told has, n (%):			--	--			--	--
Heart disease*	2 (1.7)	2 (1.2)			4 (14.3)	0		
Heart failure	3 (2.6)	0			4 (14.3	0		
Hypertension	1 (0.9)	7 (4.1)			4 (14.3)	1 (1.2)		
Diabetes	0	1 (0.6)			1 (3.6)	0		
Lung cancer	1 (0.9)	0			0	0		
Stroke*	2 (1.7)	3 (1.8)			2 (7.1)	0		
Cancer*	2 (1.8)	2 (1.2)			0	0		
Chronic kidney disease*	0	1 (0.6)			0	0		
Chronic liver disease*	0	0			0	0		
Epilepsy*	0	2 (1.2)			0	0		
Dementia*	0	1 (0.6)			0	0		
Pregnant (females only), n (%)*	1 (2.2)	3 (3.9)	--	--	0	3 (10.7)	--	--

Continued overleaf

Table 6-4 continued	HIV-positive sub-group				HIV-negative sub-group			
Exposures	**Cases (n=117)**	**Controls (n=169)**	**Unadjusted OR (95% CI)**	***P* value**	**Cases (n=28)**	**Controls (n=84)**	**Unadjusted OR (95% CI)**	***P* value**
Hospitalised as a child for malnutrition, n (%)			--	--			--	--
Yes	4 (3.4)	7 (4.1)			2 (7.1)	2 (2.4)		
No	99 (84.6)	156 (92.3)			23 (82.1)	79 (94.1)		
Don't know	14 (12.0)	6 (3.6)			3 (10.7)	3 (3.6)		
Vaccinated, n (%)*			--	--			--	--
Pneumococcal vaccine	1 (0.9)	0			0	0		
Haemophilus influenza vaccine	1 (0.9)	0			0	0		
Influenza vaccine	0	0			0	0		
Current medication other than ART/co-trimoxazole or for respiratory problems, n (%)*	11 (9.6)	21 (12.9)	--	--	9 (32.1)	11 (13.4)	--	--
Duration of ART, n (%)*‡			--	--	--	--	--	--
Less than 3 months	6 (8.8)	5 (4.1)						
3-12 months	11 (16.2)	14 (11.4)						
More than 12 months	38 (55.9)	104 (84.6)						
Don't know	13 (19.1)	7 (5.4)						

*Missing data not imputed; †per unit change; ‡ for those taking ART; § variable included in the principal components analysis for socioeconomic status score.

HIV; Human Immunodeficiency Virus; OR: odds ratio; CI: confidence interval; IQR: interquartile range; STD: standard deviation; ART: antiretroviral therapy

6.3.4. Air pollution exposure

6.3.4.1. Air pollution monitoring

Ambulatory $PM_{2.5}$ exposure data was available for 379 (95.2%) of participants who completed follow-up, while ambulatory CO, household $PM_{2.5}$, and household CO exposure data were available for 388 (97.5%), 258 (64.8%), and 375 (94.2%) of participants, respectively. Data were missing because of technical faults with the pollution monitoring devices.

Ambulatory readings were taken every 9 seconds, with an average of 47 hours 53 minutes data per participant. Household CO readings were taking every 30 seconds for 222 (59.2%) participants and every 10 seconds for 153 (40.8%) participants, with an average of 47 hours 46 minutes data per participant. Household $PM_{2.5}$ readings were taken every 1 minute, with an average of 44 hours 16 minutes data per participant.

Median duration between recruitment and ambulatory monitoring was 11 days (IQR 6-24) for controls and 65 days (IQR 57-83) for cases. Median duration between recruitment and household monitoring was 11 days (IQR 6-23) for controls and 64 days (IQR 57-78) for cases.

6.3.4.2. Measured air pollution exposures

Ambulatory and household exposures to $PM_{2.5}$ and CO are shown in Table 6-5. In the HIV-positive sub-group, ambulatory $PM_{2.5}$ and CO mean exposures were higher in the cases than the controls, but not significantly so. In the HIV-negative sub-group, mean ambulatory $PM_{2.5}$ was higher, but CO was lower, for cases than controls. For household measurements $PM_{2.5}$ mean exposures were lower and CO mean exposures were higher for cases than controls in the HIV-positive sub-group, but the reverse was true in the HIV-negative sub-group.

Ambulatory exposure to CO was significantly higher in women than in men (median mean CO exposure 5.9 ppm (women) vs 4.3 (men), $p = 0.0035$). There was no significant difference between ambulatory $PM_{2.5}$ exposures, although there was a trend towards men having higher exposures (median mean $PM_{2.5}$ exposure 57.5 (women) vs 61.2 (men), $p = 0.575$).

Table 6-5: Measured ambulatory and household air pollution exposures in HIV-positive and HIV-negative cases and controls, and univariate analysis of the association between measured air pollution exposures and pneumonia.

Exposures	HIV-positive sub-group				HIV-negative sub-group			
	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Ambulatory exposures								
Mean ambulatory $PM_{2.5}$ exposure ($\mu g/m^3$), median [IQR]	60.4 [41.0-103.0]	55.2 [34.6-89.1]	1.00 (1.00-1.00) †	0.145	70.7 [50.3-109.4]	56.7 [41.3-92.3]	1.00 (1.00-1.01) †	0.410
Mean ambulatory CO exposure (ppm), median [IQR]	6.0 [2.7-11.3	4.5 [2.5-9.2]	1.03 (1.00-1.07) †	0.047	3.1 [1.2-7.4]	4.7 [1.0-11.5]	0.93 (0.85-1.01) †	0.079
Peak ambulatory $PM_{2.5}$ exposure ($\mu g/m^3$), median [IQR]*	5749.5 [2732.1 – 13816.0]	5226.6 [2221.0 – 15190.1]	1.00 (1.00-1.00) †	0.975	19617.73 [4252.3 – 53123.5]	5294.5 [2501.5 – 14898.2]	1.00 (1.00-1.00) †	0.013
Peak ambulatory CO exposure (ppm), median [IQR]*	130.4 [77.5-203.8]	137.5 [69.6 – 237.6]	1.00 (1.00-1.00) †	0.352	121.9 [44.5 – 216.5]	137.3 [48.5 – 237.2]	1.00 (1.00-1.00) †	0.466
Highest ambulatory 15 min average $PM_{2.5}$ exposure ($\mu g/m^3$), median [IQR]*	953 [582.7 – 2134.8]	804.9 [425.9 – 1627.7]	1.00 (1.00-1.00) †	0.538	1267.3 [697.8 – 2444.2]	906.2 [436.4 – 2190.8]	1.00 (1.00-1.00) †	0.807

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Table 6-5 continued	HIV-positive sub-group				HIV-negative sub-group			
Exposures	**Cases (n=117)**	**Controls (n=169)**	**Unadjusted OR (95% CI)**	***P* value**	**Cases (n=28)**	**Controls (n=84)**	**Unadjusted OR (95% CI)**	***P* value**
Highest ambulatory 15 min average CO exposure (ppm), median [IQR]*	97.4 [146.4 – 35.8]	73.3 [40.0 – 135.5]	1.00 (1.00-1.00)†	0.562	55.1 [21.3 – 111.6]	85.7 [33.3 – 143.2]	1.00 (0.99-1.00)†	0.186
Highest ambulatory 60 min average $PM_{2.5}$ exposure ($\mu g/m^3$), median [IQR]*	473.4 [292.4 – 1174.8]	382.0 [207.1 – 780.1]	1.00 (1.00-1.00)†	0.309	544.4 [347.5 – 937.7]	397.0 [243.6 – 919.3]	1.00 (1.00-1.00)†	0.877
Highest ambulatory 60 min average CO exposure (ppm), median [IQR]*	67.4 [25.9 – 102.6]	54.7 [29.5 – 93.8]	1.00 (1.00-1.00)†	0.327	40.3 [8.3 – 76.1]	46.0 [21.5 – 106.4]	1.00 (0.99-1.00)†	0.186
Household exposures								
Mean household $PM_{2.5}$ exposure ($\mu g/m^3$), median [IQR]*	125.2 [77.4-254.9]	167.1 [90.6-311.9]	1.00 (1.00-1.00)†	0.559	189.5 [132.4-344.3]	132.4 [69.5-292.1]	1.00 (1.00-1.00)†	0.074
Mean household CO exposure (ppm), median [IQR]	6.9 [2.8-13.6]	5.4 [2.9-11.8]	1.02 (1.00-1.04)†	0.109	4.5 [2.4-8.7]	7.5 [3.6-16.1]	0.96 (0.90-1.01)†	0.121
Peak household $PM_{2.5}$ exposure ($\mu g/m^3$), median [IQR]*	3417.4 [1925.4 – 11669.6]	6170.5 [2248.2 – 16019.7]	0.99 (0.98-1.01)†	0.308	13144.8 [5992.2-32884.2]	5362.2 [2353.7 – 16210.1]	1.01 (1.00-1.02)†	0.162

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Table 6-5 continued	HIV-positive sub-group				HIV-negative sub-group			
Exposures	**Cases (n=117)**	**Controls (n=169)**	**Unadjusted OR (95% CI)**	***P* value**	**Cases (n=28)**	**Controls (n=84)**	**Unadjusted OR (95% CI)**	***P* value**
Peak household CO exposure (ppm), median [IQR]*	133.5 [58.3 – 262.8]	115.0 [62.5 – 207.5]	1.00 (1.00-1.00)†	0.131	130 [76.5-226]	155 [91.0 – 287.5]	1.00 (0.99-1.00)†	0.210
Highest household 15 min average $PM_{2.5}$ exposure ($\mu g/m^3$), median [IQR]*	1789.2 [831.5 – 3522.6]	2224.3 [828.8 – 5259.5]	1.00 (1.00-1.00)†	0.500	3678.0 [1679.7 – 14205.2]	1545.1 [706.1 – 4814.7]	1.00 (1.00-1.00)†	0.134

*Missing data not imputed; †per unit change. HIV: Human Immunodeficiency Virus; OR: odds ratio; CI: confidence interval; IQR: interquartile range; $PM_{2.5}$: particulate matter <2.5µm; CO: carbon monoxide; ppm: parts per million.

6.3.4.3. Self-reported air pollution exposures

Self-reported exposures to pollutants in the home and workplace are reported in Table 6-6. Almost all - 395 (99.2%) - participants reported that their household had frequently used at least one form of solid fuel for a period of at least six months at some point in their lifetime.

In the HIV-positive sub-group, controls tended to cook with solid fuels more frequently than cases, although only around 10% of both groups reported cooking "often" or "frequently". Type of solid fuel used was very similar between HIV-positive cases and controls, with charcoal being by far the most commonly used fuel for cooking. Most households use a portable clay cookstove. Most households did not use any form of heating, and candles and electricity were the two most common forms of lighting for households in both cases and control groups. Approximately a quarter of both cases and controls in the HIV-positive sub-group report cooking for commercial purposes and burning mosquito coils inside the home.

In the HIV-negative sub-group, cooking, heating and lighting habits of cases and controls were similar to that of the HIV-positive sub-group, although cases were less likely to use charcoal than controls (64.3% vs 81.0%) and more likely to use wood (28.6% vs 7.1%) as their primary cooking fuel.

Women reported cooking much more frequently than men ($p < 0.001$) but there were no differences between type of cooking fuel used between genders. There was no difference between men and women in heating and lighting use. Women were more likely to report smoke exposure from beer brewing (6.2% vs 1.3%, $p = 0.006$) and commercial cooking (30.4% vs 11.4%, $p < 0.001$) but men reported exposures from brick making and women did not (17.7% vs 0%, $p < 0.001$). Men were also more likely to report occupational past or current occupational exposures to smoke or dust than women (81.5% vs 63.4%, $p = <0.001$).

Table 6-6: Self-reported exposure to air pollutants in HIV-positive and HIV-negative cases and controls, and univariate analysis of the association between self-reported air pollution exposures and pneumonia.

	HIV-positive sub-group				HIV-negative sub-group			
	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Household cooking								
Cooking with solid fuel frequency, n (%)								
Cooks rarely	29 (24.8)	22 (13.0)	1	--	4 (14.3)	16 (19.1)	1	--
Cooks occasionally	36 (30.8)	53 (31.4)	0.52 (0.26-1.03)	0.062	16 (57.1)	23 (27.4)	2.78 (0.78-9.89)	0.114
Cooks sometimes	40 (34.2)	73 (43.2)	0.42 (0.21-0.82)	0.011	8 (28.6)	34 (40.5)	0.94 (0.25-3.59)	0.929
Cooks often	12 (10.3)	20 (11.8)	0.46 (0.18-1.13)	0.088	0 (0)	10 (11.9)	1	--
Cooks frequently	0 (0)	1 (0.6)	1	--	0 (0)	1 (1.2)	1	--
Types of stove used for cooking, n (%)			--	--			--	--
Open 3-stone fire	45 (38.5)	78 (46.2)			15 (53.6)	33 (39.3)		
Permanent clay cookstove	12 (10.3)	28 (16.6)			3 (10.7)	19 (22.6)		
Portable clay cookstove	96 (82.1)	132 (78.1)			21 (75.0)	62 (73.8)		
Metal cookstove	1 (0.9)	0			0	0		
Electric cooker	12 (10.3)	26 (15.4)			3 (10.7)	16 (19.1)		
Primary cooking fuel, n (%)								
Electricity	9 (7.7)	13 (7.7)	1	--	1 (3.6)	10 (11.9)	1	--
Wood	17 (14.5)	26 (15.4)	0.94 (0.33-2.69)	0.915	8 (28.6)	6 (7.1)	13.33 (1.32-134.61)	0.028
Charcoal	130 (76.5)	130 (76.9)	1.01 (0.41-2.46)	0.981	18 (64.3)	68 (81.0)	2.65 (0.32-22.06)	0.368
Plastic Bottles	0 (0)	0 (0)	--	--	1 (3.6)	0 (0)	1	--

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Table 6-6 continued	HIV-positive sub-group				HIV-negative sub-group			
	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Fuels used for cooking, n (%)			--	--			--	--
Wood	44 (37.6)	78 (46.2)			15 (53.6)	34 (40.5)		
Charcoal	107 (91.5)	160 (94.7)			24 (85.7)	80 (95.2)		
Electricity	12 (10.3)	26 (15.4)			3 (10.7)	16 (19.1)		
Plant matter / crop residue	2 (1.7)	0			0	1 (1.2)		
Kerosene	1 (0.9)	0			0	0		
Gas	1 (0.9)	1 (0.6)			0	0		
Wood shavings	0	0			0	1 (1.2)		
Plastic bottles	0	0			1 (3.6)	1 (1.2)		
Ventilation whilst cooking, n (%)								
Mainly cooks outside or only uses electricity	22 (18.8)	28 (16.6)	1	--	2 (7.14)	14 (16.7)	1	1
Mainly cooks inside with ventilation	73 (62.4)	107 (63.3)	0.86 (0.46-1.63)	0.662	19 (67.9)	66 (78.6)	2.02 (0.42-9.66)	0.381
Mainly cooks inside without ventilation	22 (18.8)	34 (20.1)	0.82 (0.38-1.79)	0.623	7 (25.0)	4 (4.8)	12.25 (1.79-83.95)	0.011

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Table 6-6 continued	HIV-positive sub-group				HIV-negative sub-group			
	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Types of fuel used regularly for at least 6 months in lifetime, n (%)			--	--			--	--
Wood	112 (95.7)	169 (99.4)			28 (100.0)	79 (94.1)		
Charcoal	98 (83.8)	157 (92.9)			22 (78.6)	66 (78.6)		
Electricity	11 (9.4)	12 (7.1)			1 (3.6)	6 (7.1)		
Plant matter / crop residue	57 (48.7)	95 (56.2)			15 (53.6)	37 (44.1)		
Kerosene	4 (3.4)	4 (2.7)			0	1 (1.2)		
Gas	0	3 (1.8)			0	0		
Wood shavings	12 (10.3)	29 (17.2)			5 (17.9)	9 (10.7)		
Animal dung	1 (0.9)	3 (1.8)			0	2 (2.4)		
Household heating and lighting								
Uses solid fuel for heat/light, n (%)								
No	105 (89.7)	154 (91.1)	1	--	23 (82.1)	78 (92.9)	1	--
Yes	12 (10.3)	15 (8.9)	1.17 (0.53-2.61)	0.695	5 (17.9)	6 (7.1)	2.83 (0.79-10.11)	0.110
Fuels used for heating, n (%)*			--	--			--	--
Wood	2 (1.7)	1 (0.6)			1 (3.6)	1 (1.2)		
Charcoal	2 (1.7)	4 (2.4)			0	0		
Electric	0	2 (1.2)			0	0		
Doesn't use heating	115 (98.3)	162 (95.9)			27 (96.4)	83 (98.8)		

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Table 6-6 continued	HIV-positive sub-group				HIV-negative sub-group			
	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Types of lighting, n (%)			--	--			--	--
Battery operated torch	25 (21.4)	34 (20.1)			8 (28.6)	17 (20.2)		
Simple paraffin lamp	6 (5.1)	1 (0.6)			0	1 (1.2)		
Hurricane lamp	3 (3.4)	8 (4.7)			4 (14.3)	3 (3.6)		
Candles	95 (81.2)	135 (79.9)			19 (67.9)	67 (79.8)		
Electricity	57 (48.7)	76 (45.0)			11 (39.3)	47 (56.0)		
Solar light	0	5 (2.9)			1 (3.6)	1 (1.2)		
Mobile phone torch	0	4 (2.4)			0	1 (1.2)		
Other exposures								
Work related dust/smoke exposure, n (%)								
Never exposed	32 (27.4)	33 (19.5)	1	--	6 (21.4)	32 (38.1)	1	--
Previous exposure	44 (37.6)	66 (39.1)	0.69 (0.37-1.28)	0.235	13 (46.4)	23 (27.4)	3.01 (1.00-9.11)	0.050
Current exposure	41 (35.0)	70 (41.4)	0.60 (0.32-1.12)	0.111	9 (32.1)	29 (34.5)	1.66 (0.52-5.22)	0.390
Number of years working in a dusty/smoky environment, median [IQR]*	5 [2-14]	5 [2-12]	--	--	10 [3-15]	4 [2-9.5]	--	--

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Table 6-6 continued	HIV-positive sub-group				HIV-negative sub-group			
	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Other smoke exposures, n (%)*			--	--			--	--
Burning rubbish	2 (1.7)	3 (1.8)			1 (3.6)	1 (1.2)		
Brewing beer	3 (2.6)	6 (3.6)			1 (3.6)	3 (3.6)		
Commercial cooking	27 (23.1)	36 (21.3)			3 (10.7)	10 (11.9)		
Making bricks	10 (8.6)	16 (9.5)			5 (17.9)	11 (13.1)		
Burning mosquito coils	31 (26.5)	46 (27.2)			6 (21.4)	18 (21.4)		
Other	3 (2.6)	3 (1.8			1 (3.6)	3 (3.6)		

*Missing data not imputed

HIV: Human Immunodeficiency Virus; OR: odds ratio; CI: confidence interval; IQR: interquartile range; STD: standard deviation.

6.3.4.4. Exhaled carbon monoxide

eCO was measured in all 145 cases and 251 (99.2%) of controls. In the HIV-positive sub-group, median eCO was 4 (IQR 2-6) and 4 (IQR 3-6) in the cases and controls, respectively. In the HIV-negative sub-group, eCO was 4 (IQR 3-6) and 3 (IQR 2-6) in the cases and controls, respectively. There was no association between eCO and pneumonia risk in either HIV-positive or HIV-negative sub-groups (OR 0.98 (95% CI 0.93-1.03, p=0.430) and OR 1.02 (95% CI 0.96-1.10, p=0.479), respectively). There was a weak but statistically significant association between eCO and mean ambulatory CO exposure (Spearman's coefficient, rs 0.113, p=0.025). There was no association between eCO and mean ambulatory $PM_{2.5}$ exposure (rs 0.061, p=0.223), mean household CO exposure (rs 0.076, p=0.119) or mean household $PM_{2.5}$ exposure (rs -0.003, p=0.968).

6.3.5. Chronic respiratory disease

Table 6-7 details the respiratory symptoms, previous respiratory diagnoses and respiratory medication use reported by cases and controls. CRD (composite definition) was more common in cases than controls (138 (95%) vs 97 (38.3%)) (Table 6-7), and amongst controls, was more common in HIV-positive individuals than HIV-negative individuals (80 (47.3%) vs 17 (20.2%)). Emphysema was the most commonly reported respiratory diagnosis, and this was more common in cases than controls in both HIV-positive and HIV-negative sub-groups. Very few HIV-positive or HIV-negative individuals had taken medications for respiratory problems in the preceding 12 months, but this was slightly more common in cases than controls for both sub-groups. Cases more commonly reported chronic respiratory symptoms than controls in both sub-groups, but symptoms were more common in HIV-positive controls than HIV-negative controls (45.6% vs 17.9).

Only four (12.9%) of the controls who reported chronic cough attended for sputum induction, and GeneXpert® was negative in all of these individuals.

Measured air pollution exposures were not associated with the presence of CRD (data not shown).

Table 6-7: Chronic respiratory diagnoses, medications and symptoms in HIV-positive and HIV-negative cases and controls, and univariate analysis of the association between past respiratory health and pneumonia.

	HIV-positive sub-group				HIV-negative sub-group			
	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Respiratory diagnosis history and medications								
Chronic respiratory disease, n (%)								
No	6 (5.13)	89 (52.4)	1	--	1 (3.6)	67 (79.8)	1	--
Yes	111 (94.9)	80 (47.3)	20.58 (8.58-49.38)	< 0.001	27 (96.4)	17 (20.2)	106.41 (13.49-839.66)	< 0.001
Previous respiratory diagnosis, n (%)								
No	14 (12.0)	103 (60.6)	1	--	2 (7.1)	31 (86.9)	1	--
Yes	103 (88.0)	67 (39.4)	11.48 (6.07-21.73)	< 0.001	26 (92.9)	11 (13.1)	86.27 (17.92-415.40)	< 0.001
Ever been told has, n (%):			--	--			--	--
Tuberculosis (in past)	30 (25.6)	36 (21.3)			4 (14.3)	1 (1.2)		
Pneumonia (as adult, in past)	37 (31.6)	40 (23.8)			9 (32.1)	3 (3.6)		
Emphysema*	100 (86.2)	50 (29.9)			24 (85.7)	9 (10.7)		
Asthma / Allergic bronchitis*	14 (12.2)	5 (3.0)			3 (10.7)	4 (4.8)		
Chronic bronchitis*	0	0			0	0		
COPD*	0	2 (1.2)			0	0		
Medication for respiratory problems in past 12 months, n (%)*	5 (4.4)	1 (0.6)	--	--	2 (7.4)	2 (2.5)		

Continued overleaf

Table 6-7 continued	HIV-positive sub-group				HIV-negative sub-group			
	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Hospitalised for breathing problems before age of 10, n (%)*			--	--			--	--
Yes	7 (6.0)	6 (3.6)			2 (7.1)	2 (2.4)		
No	88 (75.9)	145 (85.8)			19 (67.9)	73 (88.0)		
Don't know	21 (18.1)	18 (10.7)			7 (25.0)	8 (9.6)		
Self-reported chronic symptoms								
Previous chronic respiratory symptoms, n (%)								
No	8 (6.8)	92 (54.4)	1	--	1 (3.6)	69 (82.1)	1	--
Yes	109 (93.2)	77 (45.6)	16.28 (7.49-35.01)	< 0.001	27 (96.4)	15 (17.9)	124.20 (15.63-986)	< 0.001
Usually has cough when doesn't have a cold	21 (18.0)	24 (14.2)	--	--	6 (21.4)	7 (8.3)	--	--
Short of breath when hurrying on a level or walking up a slight hill*	15 (14.2)	20 (12.1)	--	--	3 (13.6)	3 (3.8)	--	--
Usually has phlegm on chest when doesn't have a cold	11 (9.4)	20 (11.8)	--	--	2 (7.1)	2 (2.4)	--	--
Wheeze during past 12 months	9 (7.7)	12 (7.1)	--	--	6 (21.4)	4 (4.8)	--	--
Breathing problems which have interfered with usual daily activities in the past 12 months	106 (90.6)	57(33.7)	--	--	27 (96.4)	6 (7.1)	--	--

*Missing data not imputed.

HIV: Human Immunodeficiency Virus; OR: odds ratio; CI: confidence interval; IQR: interquartile range; COPD: chronic obstructive pulmonary disease.

6.3.5.1. Spirometry

Two independent reviewers deemed the spirometry data as usable per ATS standards in 349 (87.7%) participants who completed follow-up. The two reviewers agreed on the spirometry interpretation for 99.1% of participants. Of the 91 (72.8%) cases that had abnormal spirometry at their initial follow-up appointment, it was only possible to repeat spirometry in 13 (14.3%) a minimum of 4 months after their pneumonia episode to determine their final spirometry status: spirometry remained abnormal in all these individuals.

Pre-bronchodilator percentage of predicted FEV_1 and FVC were lower in cases than in controls in both the HIV-positive and HIV-negative sub-groups (Table 6-8). Abnormal spirometry was identified in 91 (72.8%) cases compared to 111 (49.3%) controls. In all groups, restrictive abnormalities were more common than obstructive abnormalities: overall 150 (43.0%) individuals had restrictive spirometry compared to 52 (14.9%) with obstructive spirometry. Of those with restrictive disease, 34 (22.7%), 50 (33.3%), 54 (36.0%) and 12 (8.0%) had mild, moderate, moderately severe and severe disease, respectively. Of those with obstructive disease, 2 (3.9%), 7 (13.5%), 13 (25.0%), 20 (38.5%) and 10 (19.2%) had mild, moderate, moderately severe, severe and very severe disease, respectively.

Table 6-8: Spirometry findings in HIV-positive and HIV-negative cases and controls, and univariate analysis of the association between spirometry findings and pneumonia.

	HIV-positive sub-group				HIV-negative sub-group			
	Cases (n=117)	Controls (n=169)	Unadjusted OR (95% CI)	*P* value	Cases (n=28)	Controls (n=84)	Unadjusted OR (95% CI)	*P* value
Spirometry (using Caucasian reference ranges)								
FEV_1 % of predicted (per 1% change), median [IQR] *	60.2 [54.2-73.3]	70.3 [62.0-81.0]	0.98 (0.96-0.99) †	0.006	57.0 [41.7-66.0]	71.4 [61.6-79.7]	0.93 (0.90-0.97) †	< 0.001
FVC % of predicted (per 1% change), median [IQR] *	74.7 [65.4-82.6]	81.5 [71.7-88.6]	0.97 (0.95-0.99) †	0.001	73.0 [64.6-80.4]	82.2 [73.1-89.6]	0.93 (0.89-0.97) †	0.002
Spirometric classification, n (%)*								
Normal	28 (27.5)	76 (51.7)	1	--	6 (26.1)	37 (48.1)	1	--
Obstructive	17 (16.7)	17 (11.6)	2.71 (1.22-6.04)	0.014	8 (34.8)	10 (13.0)	4.93 (1.39-17.54)	0.014
Restrictive	57 (55.8)	54 (36.7)	2.87 (1.61 – 5.07)	<0.001	9 (39.1)	30 (39.0)	1.85 (0.59-5.78)	0.290
Spirometry (using African-American reference ranges)								
FEV_1 % of predicted (per 1% change), median [IQR] *	87.7 [75.7 – 101.9]	95.9 [85.1 – 108.0]	--	--	82.0 [69.8 – 99.6]	97.9 [88.8 – 110.4]	--	--
FVC % of predicted (per 1% change), median [IQR] *	93.3 [82.4 – 105.2]	101.4 [89.3 – 110.7]	--	--	86.2 [81.6 -101.1]	103.3 [88.9 – 111.4]	--	--
Spirometric classification, n (%)*			--	--			--	--
Normal	10 (68.6)	120 (81.1)			13 (56.5)	63 (81.8)		
Obstructive	16 (15.7)	17 (11.5)			8 (34.8)	10 (13.0)		
Restrictive	16 (15.7)	11 (7.4)			2 (8.7)	4 (5.2)		

*Missing data not imputed; †per unit change.

HIV: Human Immunodeficiency Virus; OR; odds ratio; CI: confidence interval; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; IQR: interquartile range

Using Caucasian reference ranges, abnormal spirometry was associated with the presence of CRD (composite definition) in the HIV-positive sub-group (Pearson's chi-square test= 15.22, p<0.001) but not in the HIV-negative sub-group (1.93, p=0.165). Abnormal spirometry was not associated with CRD in the control population (3.34, p=0.068). Similar findings were noted between abnormal spirometry and chronic respiratory symptoms.

Use of African-American reference ranges resulted in a higher FVC % of predicted result compared to using Caucasian reference ranges, and therefore the prevalence of restrictive airways disease was lower. Using African-American reference ranges, abnormal spirometry was associated with the presence of CRD in the HIV-positive sub-group (Pearson's chi-square test= 9.92, p=0.002) but not in the HIV-negative sub-group (3.50, p=0.061). An association was found between CRD and abnormal spirometry in the control population, if African-American reference ranges were used (6.46, p=0.011).

In the control group, there were no significant associations found between either restrictive or obstructive airways disease and BMI, socioeconomic status or ambulatory $PM_{2.5}$ and CO mean exposures (data not shown) in univariate analysis or after adjustment for age and gender.

In the control group, BMI was associated with pre-bronchodilator % of predicted FEV_1 ($p < 0.001$) and % of predicted FVC ($p = 0.001$) but there were no associations between socioeconomic status and pre-bronchodilator % of predicted FEV_1 value or % of predicted FVC value ($p = 0.185$ and $p=0.094$, respectively). There were no associations between mean $PM_{2.5}$ or CO exposures and spirometry findings in the control group.

6.3.6. Univariate analysis of potential risk factors

Findings were consistent for pollution assessment modalities in both HIV-positive and HIV-negative sub-groups: exposure to ambulatory and household $PM_{2.5}$ and CO had no effect on pneumonia risk with unadjusted ORs of approximately one for all measures of mean and peak exposures (Table 6-5). In the HIV-positive sub-group, there were no consistent findings related to frequency of cooking with solid fuels and no significant findings relating to fuel use, household ventilation, or other reported forms of pollution exposure (Table 6-6). In the HIV-negative sub-group, cooking with wood (OR 13.33 [95% CI 1.32–134.61, p=0.028]) and cooking inside without ventilation (OR 12.25 [95% CI 1.79–83.95, p=0.011]) were both associated with an increased risk of pneumonia.

CRD was associated with an increased risk of pneumonia in both study groups (HIV-positive: OR 20.58 [95% CI 8.58–49.38], p<0.001; and HIV-negative: OR 106.41 [95% CI 13.49–839.66, p<0.001]) (Table 6-7). Restrictive spirometry was a risk factor for pneumonia in the HIV-positive sub-group only (OR 2.87 [95% 1.61–5.07, p<0.001]) whereas obstructive spirometry was predictive of pneumonia in both sub-groups (HIV-positive: OR 2.71 [95% CI 1.22–6.04, p=0.014]; and HIV-negative: OR 4.93 [95% CI 1.39-17.54, p=0.014]) (Table 6-8). Similar results were found in univariate analysis of spirometric findings using African-American reference ranges (data not shown).

Factors associated with a reduced risk were taking antiretroviral treatment (OR 0.42 [95% CI 0.25–0.70, p=0.001]), increasing BMI (HIV-positive: OR 0.85 [95% CI 0.78–0.92, p<0.001]; HIV-negative: 0.84 [95% CI 0.72–0.98, p=0.023]) and increasing CD4 count (cells/µl) (OR 0.99 [95% CI 0.99–0.99, p<0.001]) (Table 6-4). In the HIV-positive sub-group, socioeconomic status was not associated with pneumonia (OR 1.00 [95% CI 0.85–1.20, p=0.943]), but there was an increased risk of pneumonia with decreasing socioeconomic status in the HIV-negative sub-group (OR 1.38 [95% CI 1.02–1.85, p=0.034]). Other potential risk factors and confounding factors are reported in Tables 6-4 to 6-8.

6.3.7. Multivariate analysis of potential risk factors

After adjustment for confounders, mean ambulatory and household $PM_{2.5}$ and CO exposures were not associated with pneumonia in the HIV-positive or HIV-negative sub-groups (

Table 6-9). CRD had a substantial effect on pneumonia risk in both HIV-positive and HIV-negative sub-groups (aOR 28.07 [95% CI 9.28–84.83 p<0.001] and aOR 104.27 [95% CI 12.86–852.35, p<0.001], respectively). Factors associated with a reduced risk of pneumonia after adjustment for confounders in the HIV-positive sub-group included BMI (HIV-positive: aOR 0.84 [95% CI 0.74–0.95, p=0.005]), increasing CD4 count (aOR 0.99 [95% CI 0.99–0.99, p<0.001]) and antiretroviral therapy (aOR 0.24 [95% CI 0.09–0.61, p=0.003]). In the HIV-negative sub-group, after adjustment for age and sex, being an ex-smoker (aOR 5.92 [95% CI 1.69–20.79, p=0.006]) and cooking inside without ventilation (aOR 9.32 [95% CI 1.24–69.81, p=0.030]) were associated with an increased risk of pneumonia. Increasing BMI (aOR 0.84 [95% CI 0.72–0.99, p=0.036]) was found to be protective against pneumonia in the HIV-negative sub-group. We did not find evidence of spatial clustering in pneumonia risk, with all p-values for the test on the presence of residual spatial effects being well above 10%.

Table 6-9: Multivariate analysis of the effects of household air pollution exposure and chronic respiratory disease on pneumonia risk for HIV-positive and HIV-negative sub-groups.

Exposures	Adjusted OR (95% CI)	*P* value
HIV-positive sub-group		
Mean ambulatory $PM_{2.5}$ exposure ($\mu g/m^3$)*	1.00 (1.00–1.01)**	0.141
Mean ambulatory CO exposure (ppm)*	1.07 (1.00–1.14)**	0.052
Mean household $PM_{2.5}$ exposure ($\mu g/m^3$) †§	1.00 (1.00–1.00) **	0.608
Mean household CO exposure (ppm)*	1.03 (1.00–1.07) **	0.081
Chronic respiratory disease*	28.07 (9.29–84.83)	< 0.001
HIV-negative sub-group		
Mean ambulatory $PM_{2.5}$ exposure ($\mu g/m^3$) ‡	1.00 (0.99–1.01) **	0.872
Mean ambulatory CO exposure (ppm)‡	0.95 (0.87–1.03) **	0.219
Mean household $PM_{2.5}$ exposure ($\mu g/m^3$)‡ §	1.00 (1.00–1.00) **	0.307
Mean household CO exposure (ppm)‡	0.96 (0.91–1.02) **	0.206
Chronic respiratory disease‡	104.27 (12.86–852.35)	<0.001

*Adjusted for age, sex, CD4, chronic respiratory disease, antiretroviral treatment, body mass index, occupational status and alcohol intake; †adjusted for age, sex, CD4, chronic respiratory disease and antiretroviral treatment; ‡adjusted for age and sex; §Missing household $PM_{2.5}$ data were not imputed; therefore, analyses were restricted to 169 and 79 observations in the HIV–positive and HIV–negative sub-groups, respectively; **per unit change.

OR: odds ratio; CI: confidence interval; HIV: Human Immunodeficiency Virus; $PM_{2.5}$: particulate matter <2.5µm; CO: carbon monoxide; ppm: parts per million.

6.4. Discussion

6.4.1. Recruitment and participant characteristics

The AIR study aimed to recruit a cohort of pneumonia patients, representative of those presenting with community acquired pneumonia to a large tertiary referral centre in urban sub-Saharan Africa, alongside a cohort of healthy controls, randomly selected from the same population that the pneumonia patients came from.

Recruitment was slower than anticipated for both cohorts. For both cases and controls, recruitment was particularly slow during the time of the two natural disasters affecting Malawi during the time of the study (floods and drought). This was presumed to be due to difficulties accessing healthcare (and

therefore a lower than usual attendance rate at the hospital) and lack of time to devote to study activities whilst dealing with the consequences of the disaster. It was also more difficult to locate participants at their households after the floods, as many patients had been forced to relocate.

Many of the screened respiratory patients were ineligible for the study due to their duration of symptoms being longer than 14 days (913, 42.5%). This possibly reflects the high prevalence of tuberculosis in this population, but may also be due to the high prevalence of CRD and difficulties in distinguishing between acute and chronic symptoms at presentation. If patients with chronic respiratory symptoms who had an acute respiratory exacerbation were mistakenly deemed ineligible, this may have biased our CRD findings towards the null; despite this a strong association between CRD and pneumonia was found. Delayed presentation, which may be related to the fact we were recruiting from a tertiary referral hospital or health-seeking behaviours, may have also contributed the high number of people excluded due to prolonged symptom duration. This may have impacted on the demography of the cohort and the severity of their pneumonia. Although efforts were made to screen all adults presenting to QECH with respiratory problems in order to capture a wide spectrum of pneumonia patients, it is likely that those with the mildest form of community acquired pneumonia (who did not warrant referral to the tertiary centre), and those with the most severe disease (who did not survive until arriving at QECH) were not recruited. Of those recruited, those with the most severe disease and poorest outcome (*i.e*. death) were not included in the final analysis because they were unable to complete follow up. However, there was little difference between those who completed the study and those who did not in terms of baseline vital signs (Table 6-3).

404 (18.8%) respiratory patients lived outside of Blantyre or moved outside of Blantyre during the course of the study and so were ineligible, as the study aimed to evaluate the effect of air pollution in an urban setting. A higher than anticipated number of recruited cases either died or were started on tuberculosis treatment prior to follow-up (179, 41.8%), resulting in a smaller case sample size than planned.

Control recruitment was challenging due to the strict quotas required to ensure that consistent numbers of individuals were recruited to each age/gender/HIV strata across the time period of study recruitment: 676 (45.3%) potential controls were ineligible as they didn't meet the requirements for stratified recruitment. This was ameliorated by introducing recruitment from antiretroviral therapy clinics at health centres, but still resulted in slower than anticipated control recruitment. In particular, it was

difficult to find young HIV-positive males as they tended to not be at home during the daytime and did not regularly attend antiretroviral therapy clinics.

Several measures were taken to ensure that the controls were recruited at random from the same population from which the cases were derived. Although these measures made recruitment more challenging, and likely resulted in a smaller sample size, this reduced the risk of bias in the control population. Both cases and controls were restricted to the same geographical area (Blantyre city) and Figure 6-2 demonstrates that a good geographical spread across the city was achieved. Control recruitment locations were selected at random (weighted by population density), and individuals were selected at random from all eligible household members (including those not home at the time of the visit), ensuring that every inhabitant of Blantyre city had an equal chance of being approached for screening. However, individuals who were unavailable to complete the screening process were more likely to be male than female (86.2% vs 13.8%) and therefore the recruited male control cohort is at more risk of bias than the female control cohort.

Reluctance to participate was relatively low in both cases and controls, with 52 (2.4%) and 64 (4.6%) of potential cases and controls, respectively, either declining to consent or withdrawing consent. Controls were more likely to withdraw consent if they were HIV-positive (6.7%) than HIV-negative (2.2%), perhaps reflecting reluctance to participate following positive HIV test result, although the impact of this was small.

6.4.2. Pneumonia patient characteristics

The characteristics of patients who had pneumonia were broadly similar to those studied in other pneumonia cohorts from sub-Saharan Africa. Approximately half of recruited cases in the AIR study were considered to have pneumonia as they had confirmed chest x-ray changes and were not started on tuberculosis treatment. There was a preponderance of male gender in those with confirmed pneumonia, which is consistent with pneumonia cohorts in other settings (6, 208, 209, 218, 287, 288). A median age of 36 (IQR 30-45) in the pneumonia cohort is also similar to that seen in other pneumonia studies in sub-Saharan Africa (6, 24, 218, 231, 241, 289), but younger than seen in well-resourced settings (208, 209, 288).

HIV prevalence amongst the hospitalised pneumonia patients (81.6%) was comparable to other to studies of pneumonia in sub-Saharan Africa (6, 218, 231, 289). Median baseline CD4 count was low and

almost 40% were not on ART: this highlights an important opportunity for reducing pneumonia burden in this population, as pneumonia risk increases with decreasing CD4 count (24).

There were high rates of confirmed tuberculosis and clinically suspected tuberculosis amongst recruited cases, despite a case definition mandating a symptom duration of 14 days or less, similar rates to that identified by the MARISO study (218). We found a higher rate of tuberculosis than seen in a Kenyan study of patients with a similar case definition (9% confirmed diagnosis) (6). This is possibly explained by the additional use of GeneXpert® MTB/RIF assays in the AIR and MARISO studies. A substantial burden of *Mycobacterium tuberculosis* has been found in several community acquired pneumonia studies in sub-Saharan Africa (6, 218, 231, 289), and this high prevalence of tuberculosis amongst acutely unwell patients is an important finding which highlights the need for early clinical suspicion of pulmonary tuberculosis in high prevalence areas even in those with an acute presentation. In the MARISO study, detection of *M. tuberculosis* was associated with treatment failure at 72 hours, delayed clinical stability and increased 30-day mortality(218). This has implications for guidelines regarding empirical treatment for community acquired pneumonia patients, including the avoidance of single agent use of fluoroquinolones which may propagate resistance in *Mycobacterium tuberculosis (290)*. Pragmatic WHO guidelines recommend the addition anti-tuberculosis medication in community acquire pneumonia patients who have not responded to 3-5 days of broad spectrum antibiotic treatment (291), although this may not be an adequate strategy as it may miss 15% of patients with culture positive tuberculosis (292). This is concerning considering the high rates of early mortality seen in tuberculosis patients (293). Improvements in rapid diagnostics for *M. tuberculosis* - such as GeneXpert® MTB/RIF assay or the Alere Determine tuberculosis lipoarabinomannan (LAM) urinary antigen assay (292, 294-296) - may help, but further randomised controlled trials regarding how to clinically manage this population are warranted (297, 298).

In all recruited cases, *Streptococcus pneumoniae* was the second most commonly detected pathogen (85, 20.1% of those with either a blood culture result or a BinaxNOW® *Streptococcus pneumoniae* urinary antigen result). Pneumococcal bacteraemia was seen in 1.3%. *Streptococcus pneumoniae* was detected in 67 (21.2%) of the participants with chest x-ray changes and microbiological diagnostic results, and 50 (24.5%) of the 204 participants with pneumonia and available microbiological diagnostic results. The prevalence of pneumococcal pneumonia is similar to that detected in the MARISO study (218), but lower than other studies in sub-Saharan Africa in which intensive microbiological diagnostics were performed (including high rates of pleural fluid aspiration culture by Scott *et al)* (6, 231). The low

bacteraemia rate may in part be due to a high proportion of patients having reported receiving antibiotic treatment prior to presentation at QECH, but may also reflect inadequate microbiological diagnostics (individuals who had already had a blood culture taken by the clinical team prior to their recruitment did not have this repeated, and the clinical result was recorded where possible) (299). Indirect effects of the widespread uptake of the infant pneumococcal vaccine may have resulted in a shift towards less-invasive non-vaccine type pneumococcal serotypes, which may also explain low bacteraemia detection rates (300).

Overall hospital mortality of recruited cases was 10.7%, but was lower if limited to pneumonia patients (7.25%), which is comparable to other studies of unselected community-acquired pneumonia patients in both well and poor-resourced settings (6, 205, 218, 288). There was no difference in in-patient mortality according to HIV status which is consistent with other studies (6, 218). Death prior to follow up occurred in 64 (14.9%) of respiratory cases (29 (14.0%) of confirmed pneumonia cases) but this is likely to be an underestimate as death following discharge was not recorded for individuals who had already been excluded for other reasons.

6.4.3. Air pollution exposure

Air pollution monitoring was conducted for all 399 participants who completed the study. For almost all participants, data were available for ambulatory $PM_{2.5}$ and CO exposures and household CO exposures but, despite multiple attempts at monitoring, we were unable to collect household $PM_{2.5}$ exposure data for 35% of participants due to technical difficulties: the UCB-PATS monitors were frequently found to be faulty, and often provided only intermittent data across a 48 hours period.

While there is no gold standard method for air pollution monitoring, measuring both ambulatory and household levels of two different major components of air pollution ($PM_{2.5}$ and CO) is likely to capture a representative picture of an individual's total exposure. Mean household $PM_{2.5}$ levels detected (all homes: median 149.5 $\mu g/m^3$, IQR 85.0–289.0 $\mu g/m^3$) and mean household CO levels (all homes: median 6.4 ppm, IQR 2.9–12.6ppm) were comparable to those detected in a previous study of urban Malawian homes (mean 150 $\mu g/m^3$, standard deviation 360 $\mu g/m^3$ and mean 6.14 ppm, respectively) (3).

The detected levels of exposure greatly exceed the levels considered safe: WHO Air Quality Guidelines recommend not exceeding 24hour-mean $PM_{2.5}$ levels of 25 $\mu g/m^3$ (196). Mean ambulatory $PM_{2.5}$ levels detected (all participants: median 59.4 $\mu g/m^3$, IQR 39.6–96.1 $\mu g/m^3$) equate to a 2.5%–5% increased risk of short-term mortality according to these guidelines. For CO exposure, the WHO recommends not

exceeding 24hour-mean levels of 7mg/m^3 (equivalent 5.68 ppm) (301): mean 48-hour ambulatory CO levels detected in this study were above this level for 45% of participants with available data. Mean CO concentrations over 1 hour should not exceed 35 mg/m^3 (equivalent 28.4ppm) yet median highest ambulatory 60 min average CO exposures were 53.5pm (IQR 23.6-100.8). Furthermore, mean CO concentrations over 15 minutes should not exceed 100 mg/m^3 (equivalent 81.1ppm), but highest ambulatory 15 min average CO exposures were greater than this for 53% of those with available data (median 80.7ppm (IQR 34.4-135.8)).

Cooking was the predominant source of pollution exposure reported in this study, with only a minority of participants reporting exposures from heating, lighting, current occupational exposures, current tobacco smoke exposure or other sources. Almost all participants reported that their household had frequently used at least one form of solid fuel for a period of at least six months at some point in their lifetime. Higher ambulatory CO exposures in women compared to men is consistent with the fact women cooked more frequently than men ($p<0.001$) and the most commonly used fuel was charcoal, which emits relatively large amounts of CO when burned. Although not statistically significant, higher $PM_{2.5}$ exposures in men compared to women may be a result of their increased exposures from occupational sources. Brick making, which typically uses petrol for fuel resulting in CO emissions, was only reported by men. Burning of mosquito coils, reported by both men and women, can result in high $PM_{2.5}$ and carcinogenic polycyclic aromatic hydrocarbons associated with respiratory symptoms (58).

These high levels of exposure, well in excess of international safety standards, highlight the need to address air pollution levels in Malawi. Whilst it is not possible from our ambulatory data to distinguish between indoor and outdoor exposures, the high $PM_{2.5}$ and CO levels detected in households suggests that household air pollution is a significant contributor to this problem. With almost all households reporting use of solid fuels for cooking, but only a minority of household using heating or lighting, it is cooking practices that offer the biggest opportunity for addressing household air pollution.

6.4.4. Exhaled carbon monoxide

The need for a biomarker of exposure was discussed in Chapters 4 and 5. eCO was proposed as a potentially feasible biomarker to be used in the field in resource-poor settings. eCO measurement was well tolerated and easy to perform in this study. However, with only a very weak association between eCO and ambulatory CO exposure, and no association with the other measured pollutants parameters, there is no evidence from this study to support eCO as a biomarker of household air pollution exposure.

6.4.5. Chronic respiratory disease

A large proportion of individuals reported chronic respiratory symptoms or previous diagnoses, and so met our composite definition of CRD. The very high rate of reported symptoms in cases (93.2% of HIV-positive and 96.4% of HIV-negative) may reflect recall bias as individuals who have recently had pneumonia may be more likely to respond positively to symptom questions (although cases were asked to recall the period in the months prior to their pneumonia episode). However, the substantial rate of chronic respiratory symptoms in the HIV-positive controls (46%), suggests that there is a significant background level of respiratory problems in this population. The results for the composite definitions of chronic respiratory symptom and CRD were largely driven by individuals who reported episodes of respiratory problems interfering with their daily activities in the past 12 months (up to 7 episodes) which may be the result of acute episodes rather than chronic problems. If this is removed from the composite definition, both CRD and chronic respiratory symptoms remain strongly associated with pneumonia in both HIV-positive and HIV-negative sub-groups (CRD: HIV-positive OR 10.8 (95% CI 5.5-21.1, p <0.001) and HIV-negative OR 38.3 (95% CI 10.2 – 143.7, p <0.001); chronic respiratory symptoms: HIV-positive OR 1.6 (95% CI 1.0-2.8, p = 0.045) and HIV-negative OR 8.5 (95% CI 3.2-23.1, p <0.001)).

Chronic cough was the most commonly reported symptom, which, as noted elsewhere, may reflect undiagnosed tuberculosis (41). However, tuberculosis diagnostics were negative in 120 (82.8%) of cases and in all four of the controls with chronic cough who attended for sputum induction. It was not possible to exclude tuberculosis in 87% of the controls with a chronic cough who did not attend for sputum induction despite multiple invitations.

Emphysema was the most commonly reported previous respiratory diagnosis, whereas COPD was only reported by very few. This may reflect the terminology used by health care professionals in Malawi, or may be related to the translation of these diagnoses into Chichewa. The translated Chichewa questionnaires used to determine air pollution exposures and respiratory history and symptoms were approved by the BOLD study, to ensure that high quality translations were used and also to allow comparability with the BOLD multinational studies. However, by choosing to use externally approved translations, this prevented the opportunity to test and adapt the questionnaires locally to ensure that terminology was correctly understood by the study population, which may have impacted on our questionnaire findings and may partly explain the high prevalence of CRD identified.

For the primary analysis, spirometry was interpreted using NHANES III Caucasian reference ranges in accordance with BOLD study standards, to allow comparison of data with the multinational BOLD studies (121). When using spirometry reference ranges derived from African-American populations, the rates of restrictive disease were much lower compared to standards for Caucasian populations. This is consistent with the BOLD study from Nigeria, in which the prevalence of restriction was 70.4% and 72.8% in men and women, respectively, using NHANES III Caucasian reference ranges, compared to 17.8% and 14.4% using NHANES III African-American reference ranges, and <4% in both men and women using locally derived reference ranges (302). The previous BOLD study from Blantyre also found a prevalence reduction from 38.6% to 9% when using locally derived reference ranges compared to NHANES III Caucasian reference ranges (41). Although neither of these locally derived reference ranges have been externally validated, these findings support existing evidence that sub-Saharan Africa populations have smaller lungs than Caucasian populations (303), possibly due to the life course effects on lung health discussed below. Although genetic differences in body shape related to ethnicity are known to play a role in lung function, environmental factors are also important making choice of appropriate reference range for any given population a controversial issue (303, 304). Burney and Hooper conclude from a study of 4000 white and African-American adults in the USA that although there are differences in ventilatory function between ethnic groups, there is no evidence that there is any difference in mortality between ethnic groups for any given FVC and therefore ethnically adjusted reference ranges should not be used when assessing prognosis (305).

Using NHANES III Caucasian reference ranges, the AIR study and an earlier BOLD study in Malawi both found a high prevalence of restrictive lung disease, rather than obstructive lung disease (41). This is in keeping with other studies in sub-Saharan Africa (306) and other resource poor settings (307, 308). Low FVC has been shown to be associated with poverty (measured by Gross National Income and using proxy indicators for socioeconomic status, such as education level) (302, 307, 309). There is also evidence for an association between low BMI and reduced FVC (41, 302, 310), although the association may be non-linear and influenced by percentage body fat (311). Early life course events, such as low birthweight, childhood malnutrition and childhood respiratory infections may affect lung growth resulting in reduced adult lung function (312-316). However, a recent study from Blantyre found no association between severe acute malnutrition in early childhood and subsequent reduced lung function (317). In the AIR study, an association was found between BMI and lung function in the controls (unadjusted for confounders) and but there was no association between socioeconomic status and lung function. In

keeping with findings from Nigeria, we found no association between household air pollution and low FVC (302).

Low FVC is associated with increased mortality in other settings, even in the absence of respiratory symptoms (307, 318). Restrictive airways disease predicts mortality better than obstructive disease, and restrictive disease is associated with COPD mortality, indicating that many "COPD" deaths may in fact be due to underlying, undiagnosed restrictive disease (307). The relationship identified by AIR between airways restriction and pneumonia is potentially relevant to our understanding of this increased mortality. The underlying aetiology, pathology, epidemiology and prognosis of this low FVC phenomenon requires further investigation.

Previous studies found an association between biomass exposure and reduced FEV_1 and FVC, resulting in obstructive lung disease (319), but recent studies in sub-Saharan Africa have not found an association (41, 306, 320). In our study, no association was found between ambulatory mean $PM_{2.5}$ and CO exposures and % predicted FEV_1 or FVC; this is consistent with a study from Guatemala in which there was no association between 48 hour ambulatory CO exposure and lung function (321).

Lung function was not consistently associated with our CRD or respiratory symptoms, which may reflect our composite definitions for CRD and chronic respiratory symptoms. For example, breathlessness may be explained by cardiac dysfunction rather than respiratory disease. Inaccurate recall of symptoms or diagnoses may have also contributed to inaccurate exposure classification. The lack of association may also be explained by the true presence of abnormal lung function in the absence of respiratory symptoms, and vice-versa (322).

6.4.6. Risk factors for pneumonia

We found no association between household air pollution exposure, measured using ambulatory and household monitoring of pollutants, and radiologically confirmed pneumonia in urban Malawian adults. This was consistent across both HIV-positive and HIV-negative individuals and when measuring $PM_{2.5}$ and CO. In contrast, we found a strong association between CRD, as defined by participant-reported symptoms and diagnoses, and pneumonia in this setting. This was supported by an association between pneumonia and both restrictive (in the HIV-positive sub-group only) and obstructive lung disease, diagnosed by spirometry testing. As no association was detected between measured air pollution exposures and abnormal lung function or CRD, there is no evidence from this study that household air pollution is indirectly associated with pneumonia as a result of increased pneumonia risk from CRD.

It seems likely that socioeconomic factors explain the unexpected findings of reduced risk of pneumonia in current smokers and consumers of alcohol, as in the context of urban Malawi, the poorest individuals cannot afford cigarettes and alcohol (323). The low prevalence of smoking in this setting means that smoking is not a major driver of pneumonia risk in multivariate analysis, unlike in higher resourced countries (208).

Reduced BMI, an indicator for malnutrition (324), was a strong predictor for pneumonia in both sub-groups, even after adjustment for age, gender, CRD and CD4 in the HIV-positive sub-group: malnutrition may play a role in pneumonia risk, as has been shown in children (325). Further research into nutritional status in this population and possible interventions is warranted.

The lack of association between household air pollution and ALRI in adults identified in this study may be explained by the overwhelming effect of other major risk factors (such as CRD, HIV-associated factors and BMI), in this setting. This could explain why an association is evident in children but not adults, in whom lifelong exposure to other factors plays a more important role (12). Alternatively, it is possible that we have not detected a true association between household air pollution and ALRI in adults owing to methodological limitations, in particular with exposure assessments.

6.4.7. Strengths and weaknesses

The AIR study is the only study of household air pollution and pneumonia in adults to have used multiple measurements of air pollution exposure with radiologically confirmed hospitalised pneumonia cases. Although the largest and most detailed study of household air pollution and ALRI in adults to date, it has a number of limitations.

We were unable to evaluate HIV-positive and HIV-negative individuals together due to a lack of statistical power, but our findings were broadly consistent across both groups. The original sample size was not met because recruitment was slower than anticipated leading to lower power to detect an effect. However, in the HIV-positive group, we were able to detect an OR greater than 1.0003 per unit change for ambulatory $PM_{2.5}$ exposure (our primary exposure of interest) with 80% power. The decision to stop recruitment (at the end of the planned study period, but with a smaller than planned sample size) was made on the basis of the results of an interim analysis. There are risks of undertaking an interim analysis, especially if this has not been pre-specified in the original protocol. Firstly, the interim analysis makes the assumption that all subsequent data collected will be similar to that in the existing dataset. If future data collected differs significantly from the initial dataset, either due to systemic bias

or chance, then the predictions of the interim analysis will be inaccurate for the final analysis. Interim analysis introduces the risk of bias to the study as, for example, participant selection or exposure and outcome measurements may be influenced by the knowledge of the interim analysis findings if the study team is not blinded. There are also risks associated with repeated significance testing or multiple comparisons, which increases the probability of a Type I error. Finally, if the same rigour of data cleaning and statistical methodology is not applied at the interim analysis as compared with the final analysis, then the interim analysis will have poor predictive capabilities for determining the likely final outcome. The interim analysis for the AIR study was undertaken despite these known risks due to practical and logistical reasons: it was not possible to extend the planned recruitment period (due to financial and time constraints) unless there was a good scientific basis for doing so. The interim analysis had determined a high likelihood of detecting an association between pneumonia and CRD and ambulatory mean CO exposure at final analysis, but no association between pneumonia and ambulatory mean CO exposure was found. Reasons for the difference between the interim and final analysis findings could be that there was a difference between the initial and final datasets, or because the data used in the interim analysis had not been cleaned using the same methods as the final dataset. Given the smaller than originally planned sample size, this raises the possibility of a Type 2 error, which is the major weakness of this study.

Due to the case-control design of the study, assessment of exposures was conducted after the occurrence of the pneumonia. This resulted in the most severe cases of pneumonia (patients with the highest mortality risk) not being included in the final analysis, as they did not survive until follow-up. The potential effect of this selection bias is to bias the findings towards the null. However, we found minimal difference in baseline vital sign observations (which may indiciate the severity of illness at presentation) between cases who completed follow up and those who didn't, suggesting there were not major differences in disease severity between the two groups. In addition, our study design precluded the inclusion of individuals with milder disease, as we only recruited individuals who were admitted to QECH. We did not include individuals who may have pneumonia who did not seek healthcare, who only attended district/community health centres or who were well enough to be discharged from QECH emergency room. The potential effect of this may have been to bias the findings away from the null. This effect could have been mitigated by also recruiting from community health centres, but this was not possible due to resource contraints, such as lack of availability of x-ray machines.

Potential risk factors were assessed after the episode of pneumonia, and so questionnaire assessments may have been subject to recall bias. In particular, our composite assessment of CRD may have been vulnerable to recall bias, as cases may have been more likely to report respiratory symptoms and diagnoses than controls due to their recent illness. Spirometry results provide objective evidence of an association between CRD and pneumonia but abnormal lung function in the cases may have been a result of their acute illness rather than chronic disease (although lung function should have recovered 2 months following the acute episode). Attempts were made to account for this by repeating spirometry at least another 2 months later (and no significant changes between these two measurements were noted), but this was only possible in 14% of cases with abnormal spirometry.

Objective measurements of air pollution exposures were made, but these may not be representative of pre-pneumonia exposures, although 138/142 (97.2%) cases reported that they had returned to normal levels of function. A sensitivity analysis, in which cases without reported full functional recovery were excluded, also found no effects of mean ambulatory $PM_{2.5}$ exposure (unadjusted OR 1.00 [95% CI 1.00–1.00, p=0.113] and unadjusted OR 1.00 [95% CI 1.00–1.01, p=0.359] in the HIV-positive and HIV-negative sub-groups, respectively). In addition, our exposure monitoring does not account for differences in exposure over the course of a lifetime. The ambulatory pollutant monitoring is unable to distinguish between outdoor and indoor exposures although since our findings are consistent across ambulatory, household, and questionnaire assessments, we argue that our findings are reflective of the effects of household air pollution.

As this was an observational study, the associations we have found between exposures and pneumonia cannot be attributed as causal. Larger studies, to confirm these associations explore the role of CRD and reduced BMI in pneumonia risk in sub-Saharan Africa are warranted.

6.4.8. Conclusions

The latest Global Burden of Disease Study (2013) estimates for the burden of adult ALRI caused by household air pollution are based on data extrapolated from evidence for tobacco smoke and outdoor air pollution (9). The systematic review presented in Chapter 3 concluded that there was insufficient evidence for an association between household air pollution and pneumonia. Our findings in the AIR study are inconsistent with evidence presented by Ezzati *et al.* who demonstrated a dose-dependent relationship between household air pollution exposure and ALRI (265). This study from rural Kenya conducted household monitoring prospectively over a 2-year period (12 hours per day) but did not use

radiologically confirmed pneumonia, did not account for HIV status, and their cohort included children over the age of 5 years.

Pneumonia is a major health burden in sub-Saharan Africa (9). Although there are compelling reasons for tackling household air pollution (2), other issues need to be addressed to reduce the burden of pneumonia in adults. Evidence from this study can be used to establish global estimates for the contribution of household air pollution exposure to the burden of disease, to ensure the limited available resources for public health interventions are appropriately directed. Risk factors associated with pneumonia in this study, such as HIV and BMI, are typically associated with socioeconomic status, indicating that poverty is an important driver of pneumonia in urban African adults. To reduce the burden of pneumonia, further research into the effects of CRD and the underlying aetiologies in this setting are required. Prenatal and childhood malnutrition may play a role. Targeted evidence-based strategies to reduce the high burden of CRD seen in young adults are needed and may help to tackle the high morbidity and mortality caused by pneumonia.

7. Discussion

7.1. Thesis summary

Pneumonia causes a large burden of disease worldwide (almost 300 million cases of lower respiratory tract infection globally per year (16)), and in sub-Saharan Africa there are 200,000 deaths in adults from pneumonia annually (19). Whilst risk factors for pneumonia in developed countries are well understood, this is not the case in sub-Saharan Africa. Despite the high burden of pneumonia, little research on the topic has been conducted. Household air pollution exposure, which many individuals living in poverty worldwide face on a daily basis, is thought to cause half a million pneumonia deaths in children each year (12). In adults, estimates of attributable risk of ALRI (including pneumonia) from household air pollution exposure in the Global Burden of Disease Study rely on data extrapolated from studies of tobacco smoke and outdoor air pollution (7, 11). This thesis explores the role that household air pollution and other potential risk factors play in adult pneumonia in resource-poor settings such as Malawi, while addressing methodological challenges related to conducting household air pollution research.

After a general review of the literature (Chapter 2), I presented the findings of a systematic review of the literature, summarising the existing evidence for an association between household air pollution and ALRI in adults (Chapter 3) (283). Following systematic searches of 10 databases and other sources, which identified nearly 5000 records, only 8 studies examining the relationship between domestic use of solid fuels and ALRI in adults were found reflecting a limited body of research on the topic given the high disease burden estimated by the Global Burden of Disease Study. The heterogeneity in methods in the studies identified precluded meta-analysis and the majority of studies identified had major methodological flaws resulting in substantial risk of bias. We concluded that there was limited evidence in the existing literature for an association between household air pollution and ALRI risk in adults.

In light of the significant methodological challenges identified by the systematic review, particularly regarding exposure assessment, this thesis sought to develop tools to facilitate household air pollution research. In pilot work conducted in the UK and Malawi, two potential biomarkers of household air pollution exposure were evaluated. Firstly, using induced sputum samples collected from patients with chronic respiratory diseases in the UK, we evaluated the feasibility of using AMPL as a biomarker of exposure (Chapter 4) (326). Comparison of two digital analysis software packages for quantifying AMPL revealed that neither technique provides a suitable method for determining exposure in resource-poor

settings, due to demand on resources, expertise involved, unreliability of the automated estimates and time required. Although we were unable to comment on the accuracy of AMPL as a biomarker of exposure, we recommended that resources instead be directed towards developing a biomarker that can easily be deployed in resource-poor settings, to enable household air pollution researchers to improve exposure assessment. The second potential biomarker of exposure evaluated in this thesis was eCO. Unlike AMPL, eCO proved to be easy to employ – for both researchers and participants – when used in both rural and urban settings in Malawi (Chapters 5 & 6) (279). When eCO levels were measured in conjunction with 48 hours of detailed exposure monitoring in 397 participants of a case-control study, we found only a weak association between eCO and ambulatory CO exposure, and no association with household CO, ambulatory $PM_{2.5}$ or household $PM_{2.5}$ exposures. We therefore concluded that there is insufficient evidence to support eCO as a biomarker of exposure to household air pollution. Similar exploration of other potential biomarkers should be undertaken, until this methodological limitation of household air pollution research has been addressed.

To further address the evidence gap identified through the systematic review described in Chapter 3, this thesis went on to present the findings of a case-control study evaluating the risk factors for pneumonia in Malawian adults, with a particular focus on the role of household air pollution (Chapter 6). Hospitalised patients with radiologically-confirmed pneumonia and community controls underwent 48 hours of ambulatory and household $PM_{2.5}$ and CO exposure monitoring. In addition, participants completed questionnaires and spirometry assessments, to detect the presence of CRD. Multivariate logistic regression, stratified by HIV infection status, explored associations between these and other potential risk factors with pneumonia. Follow up was completed by 145 (117 HIV-positive; 28 HIV-negative) cases and 254 (170 HIV-positive; 84 HIV-negative) controls. Although we did not reach our intended sample size, which increases the risk of a type II error, the actual sample size was sufficient to detect an OR greater than 1.0003 per $\mu g/m^3$ change for ambulatory $PM_{2.5}$ exposure (our primary exposure of interest) with 80% power (this equates to an ability to detect an OR of 1.01 for a $20\mu g/m^3$ increase for ambulatory $PM_{2.5}$ exposure with 80% power). Exposure to high levels of household air pollution, well above WHO recommended levels, was widespread. However, we found no association between household air pollution and pneumonia in this population. This finding is in contrast to the results of the cohort study conducted by Ezzati *et al*. in Kenya (265), identified by our systematic review as being the highest quality existing evidence from sub-Saharan Africa. This may be because household air pollution exposures are different in urban Blantyre compared to rural Kenya, where multiple Maasai people may live inside the same hut alongside a continually smouldering fire. Furthermore, ALRI

diagnosis in the Kenyan study did not include radiological confirmation of pneumonia and so perhaps included alternative diagnoses and milder disease compared to the AIR study. However, there are several reasons why the null finding in the AIR study may be inaccurate, in particular: exposure assessments may have been biased as they were conducted after the episode of pneumonia in cases; exposure assessments were relatively short in the context of a lifetime of household air pollution exposure; and due to widespread high exposures (with relatively little variation in exposure between individuals) it is difficult to detect a difference between cases and controls with a small sample size. Several of the study design limitations resulted from the case-control design of the study, including retrospective exposure assessment and exclusion of cases in whom pneumonia was fatal. These limitations could be avoided in the future by using a cohort study design, although this would require substantial financial resource to allow for sufficient sample size and length of follow up. Given the high prevalence of household air pollution exposure and pneumonia in settings like Malawi, and therefore the potentially high attributable risk of household air pollution for pneumonia, investment of resources in cohort studies to clarify this association is warranted.

In contrast to the air pollution findings, CRD was strongly associated with pneumonia risk. Contrary to previous widely accepted views of CRD in sub-Saharan Africa, our study supports findings from other recent studies in Malawi that restrictive airways disease, rather than obstructive airways disease, is the predominant issue (when using the NHANES III Caucasian or locally derived reference ranges) (41). Low FVC is known to be associated with poverty and low BMI, and it is possible that early life insults such as malnutrition (including in utero) result in restricted lung growth in sub-Saharan African (302, 307, 310, 312), but there are few data regarding the aetiology, pathogenesis or prognosis of restrictive airways disease in this setting. In other settings, low FVC is known to be associated with early mortality (307, 318). If the same is true in sub-Saharan Africa, the identified association with pneumonia may partly account for this. Further research to understand the aetiology and risk factors for restrictive airways disease in sub-Saharan Africa may help to reduce the mortality burden in this setting.

7.2. Conclusions

The evidence presented in this thesis does not support an association between household air pollution and pneumonia in adults. Nevertheless, household air pollution and pneumonia both remain significant challenges in many of the poorest countries of the world. We found that pneumonia results in significant mortality in a young population in Malawi (16% of suspected pneumonia cases died prior to follow up), and that there is an underlying association with chronic respiratory illness even in this young

population. We also found that household air pollution levels are well above recommended guidelines in the homes of many ordinary Malawians, and this is associated with a wide range of health, economic, environmental and social problems as discussed in Chapter 2. This thesis has highlighted many of the methodological challenges associated with researching the effects of household air pollution in resource poor settings. To ensure that accurate conclusions are drawn from future research, and hence public health resources are appropriately allocated, it is essential that sufficient attention is given to the methodological design of household air pollution studies. In particular, accurate exposure assessment should be a priority and in settings where exposure to household air pollution is almost universally high, the sample size must be adequate to detect small changes in exposure. Two previously proposed biomarkers of exposure have been deemed unsuitable by pilot studies presented in this thesis: currently there is no adequate biomarker of exposure suitable for use in resource poor settings. Building on the findings of this thesis, recommendations for future research are outlined below.

7.3. Future research priorities

Conclusions from this thesis give rise to important questions which should be addressed by the scientific community. In this section, I outline some of the factors for consideration with regards to the following questions:

7.3.1. What is the burden of disease in adults caused by household air pollution exposure, and how can this be reduced?

The findings of the AIR study do not support the Global Burden of Disease estimates regarding the increased risk of pneumonia in adults resulting from household air pollution. Due to the methodological limitations of the AIR study that have been discussed, as well as the inconclusive evidence detailed in the systematic review, further research should be undertaken to clarify these global disease burden estimates. Robust prospective studies, including case-control studies but ideally large cohort studies, examining this association should be conducted in other locations, such as south Asia and Latin America, as the findings of the AIR study may not be generalizable to other settings where air pollution exposures and risk factor profiles (including HIV prevalence) are different. Investment in cohort studies is warranted given the potentially large burden of disease attributable to household air pollution, and data obtained from cohort studies will play an important role in achieving several of the United Nation's Sustainable Development Goals (in particular good health and well being, affordable and clean energy, reduced inequalities and climate action) (327). Furthermore, the widely accepted association between household air pollution and COPD has been challenged by recent evidence, including that from the AIR

Study. This must be clarified to ensure that burden of disease estimates are accurate and resources are appropriately allocated.

To enable such research to be undertaken to an adequate standard, attention must be focused on the methodological challenges identified in this thesis. Identification of an accurate and feasible biomarker would help improve exposure assessments, and more pilot studies of alternative potential biomarkers should be conducted before testing in larger studies is undertaken.

Efforts should continue to identify suitable interventions that are not only able to significantly reduce exposures, but that are also appropriate for the context in which they are being implemented. Recent evidence has suggested that the scientific community should broaden its focus from cookstoves, and explore other possible solutions such as fuel diversification or community level interventions (143).

7.3.2. How can the burden of pneumonia in sub-Saharan Africa be reduced?

HIV-associated factors, and other poverty-linked risk factors identified in this study (such as CRD and malnutrition), should be incorporated into the research agenda towards reducing the burden of pneumonia in sub-Saharan Africa. The role of CRD and malnutrition requires further clarification, using robust studies with validated exposure measures, and larger studies of household air pollution should be conducted to confirm or refute the findings of AIR study. RCTs of multi-faceted interventions - for example, nutritional supplements, treatment of underlying CRD, and pollution reduction measures - should be conducted to establish the most effective way of reducing exposure to confirmed risk factors, with the aim of reducing pneumonia incidence. A major challenge faced is how to address the life-course effect of multiple exposures within the context of time-limited research studies.

7.3.3. What is the impact of chronic respiratory disease in sub-Saharan Africa?

A major finding of this thesis is the important role that CRD plays in pneumonia risk in Malawian adults. Emerging evidence, from the AIR Study and other sub-Saharan African studies, suggests that restrictive disease is more prevalent than obstructive airways disease in this setting (41, 302). Although known to be associated with poverty (307), and data from Nigeria shows an association with age, gender, height, and extremes of BMI (302), little work has been done to explore low FVC in sub-Saharan Africa. Prospective longitudinal work to understand and describe the epidemiology, pathology, underlying aetiologies, burden and prognosis are required. It is likely that multiple insults throughout the life course - including maternal malnutrition, childhood respiratory infections and HIV - are implicated.

7.4. Public health and policy implications

This thesis provides data to address identified gaps in the evidence. Previously, due to lack of available data for the direct effects of household air pollution on pneumonia in adults, global estimates were based on extrapolated data from ambient air pollution and tobacco smoke exposure studies. These analyses can now be updated to improve the accuracy of disease burden estimates, and the attribution of risk factors. This will allow decision makers in low- and middle-income countries, where funds and infrastructure are scarce, to develop policies to allow the appropriate allocation of resources to tackle morbidity and mortality. In the AIR Study, CRD was strongly associated with pneumonia, with an OR of almost 30 in HIV-positive individuals and over 100 in HIV-negative individuals. This highlights the important role that the increasing burden of non-communicable diseases has on infectious diseases in vulnerable populations where HIV prevalence is high. Strategies need to be developed to tackle the epidemic of CRD in sub-Saharan Africa, but perhaps with a shift in focus towards restrictive rather than obstructive airways disease. Understanding and addressing the risk factors for low FVC has the potential to have a substantial impact on both the direct burden of disease caused by restrictive airways disease, and the subsequent risk of pneumonia. Reducing this disease burden would benefit some of the world's most vulnerable populations, a step towards tackling global health inequality.

8. References

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9. Appendix 1 – Liverpool Quality Assessment Tool

STUDY ID		EXTRACTED BY		EXTRACTION DATE	DD	MM	YY

METHODOLOGICAL QUALITY APPRAISAL

Exposure to Indoor Air Pollution and Pneumonia: A systematic review and meta-analysis

CROSS SECTIONAL STUDIES

PART A: Study Sample

QUALITY 1 – SELECTION BIAS

Is there evidence of selection bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

QUALITY 2 – RESPONSE BIAS

Is there evidence of response bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

PART B: Exposure Assessment

QUALITY 3 – Indirect Exposure

Ranking of exposure measurement?	
Poor (Uncertain discrimination)	
Adequate	
Good (clearly described, good discrimination)	*

fuel, stove, structural, behavioural

QUALITY 4 – Direct exposure

Ranking of exposure measurement?	
No direct measurement	
Indoor pollution	*
Personal child exposure	**

personal, room, calibration, how?

QUALITY 5 – RECALL BIAS

Is there evidence of recall bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

QUALITY 6 – MEASUREMENT BIAS

Is there evidence of measurement bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

PART C: Outcome Assessment

QUALITY 6 – Assessment of pneumonia

Ranking of outcome assessment?	
Parent recall	
Fieldworker assessed	*
Physician assessed	**
Radiological	***

Objective to subjective

QUALITY 7 – BIAS IN ASCERTAINMENT

Is there evidence of ascertainment bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

PART D: Analysis of Results

QUALITY 8 – ADJUSTMENT FOR CONFOUNDING

Is there adjustment for confounding?	
No	Limited or no adjustment for confounding.
Yes - adequate	The main confounders adjusted for (*)
Yes - good	Majority of known confounders in model (**)

PART E: Qualifying comments

QUALITY 8: indicate overall assessment and specific issues that you would like to draw attention to:

Total Stars	/13

STUDY ID		EXTRACTED BY		EXTRACTION DATE	DD	MM	YY

METHODOLOGICAL QUALITY APPRAISAL

Exposure to Indoor Air Pollution and Pneumonia: A systematic review and meta-analysis

CASE-CONTROL STUDIES

PART A: Study Sample

QUALITY 1 – CASE SELECTION BIAS

Is there evidence of selection bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

QUALITY 2 – CONTROL SELECTION BIAS

Is there evidence of response bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

PART B: Exposure Assessment

QUALITY 3 – Indirect Exposure

Ranking of exposure measurement?	
Poor (Uncertain discrimination)	
Adequate	
Good (clearly described, good discrimination)	*

QUALITY 4 – Direct exposure

Ranking of exposure measurement?	
No direct measurement	
Indoor pollution	*
Personal child exposure	**

QUALITY 5 – RECALL BIAS

Is there evidence of recall bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

QUALITY 6 – MEASUREMENT BIAS

Is there evidence of measurement bias?	
Yes	
Possible	
No	*
Not applicable	
If Yes/possible, provide details:	

PART C: Outcome Assessment

QUALITY 6 – Assessment of pneumonia

Ranking of outcome assessment?	
Parent recall	
Fieldworker assessed	*
Physician assessed	**
Radiological	***

PART D: Analysis of Results

QUALITY 7 – ADJUSTMENT FOR CONFOUNDING

(Including matching at design stage)

Is there adjustment for confounding?	
No	Limited or no adjustment for confounding.
Yes - adequate	The main confounders adjusted for (*)
Yes - good	Majority of known confounders in model (**)

PART E: Qualifying comments

QUALITY 8: indicate overall assessment and specific issues that you would like to draw attention to:

Total Stars	/13

STUDY ID		EXTRACTED BY		EXTRACTION DATE	DD	MM	YY

METHODOLOGICAL QUALITY APPRAISAL

Exposure to Indoor Air Pollution and Pneumonia: A systematic review and meta-analysis

COHORT STUDIES

PART A: Study Sample

QUALITY 1 – SELECTION BIAS

Is there evidence of selection bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

QUALITY 2 – RESPONSE BIAS

Is there evidence of response bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

QUALITY 3 – BIAS IN FOLLOW-UP

Is there evidence of bias in follow up?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

PART B: Exposure Assessment

QUALITY 4 – Indirect Exposure

Ranking of exposure measurement?	
Poor (Uncertain discrimination)	
Adequate	
Good (clearly described, good discrimination)	*

QUALITY 5 – Direct exposure

Ranking of exposure measurement?	
No direct measurement	
Indoor pollution	*
Personal child exposure	**

QUALITY 6 – MEASUREMENT BIAS

Is there evidence of measurement bias?	
Yes	
Possible	
No	*
Not applicable	
If Yes/possible, provide details:	

PART C: Outcome Assessment

QUALITY 6 – PNEUMONIA ASSESSMENT

Ranking of outcome assessment?	
Parent recall	
Fieldworker assessed	*
Physician assessed	**

QUALITY 7 – BIAS IN ASCERTAINMENT

Is there evidence of ascertainment bias?	
Yes	
Possible	
No	*

Radiological	***

If Yes/possible, provide details:	

PART D: Analysis of Results

QUALITY 8 – ADJUSTMENT FOR CONFOUNDING

Is there adjustment for confounding?	
No	Limited or no adjustment for confounding.
Yes - adequate	The main confounders adjusted for (*)
Yes - good	Majority of known confounders in model (**)

PART E: Qualifying comments

QUALITY 9: indicate overall assessment and specific issues that you would like to draw attention to:

Total Stars	/13

STUDY ID		EXTRACTED BY		EXTRACTION DATE	DD	MM	YY

METHODOLOGICAL QUALITY APPRAISAL

Exposure to Indoor Air Pollution and Pneumonia: A systematic review and meta-analysis

INTERVENTION STUDIES/ TRIALS

PART A: Study Sample

QUALITY 1 – SELECTION BIAS

Is there evidence of selection bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

QUALITY 2 – RESPONSE BIAS

Is there evidence of response bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

QUALITY 3 – Allocation of Intervention

Is there evidence of bias in allocation of intervention?	
Yes	
Possible	
No (but quasi-random)	*
No (randomised)	**
If Yes/possible, provide details:	

QUALITY 4 – FOLLOW-UP BIAS

Is there evidence of follow-up bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

PART B: Exposure Assessment (for intervention)

QUALITY 4 – Indirect Exposure

Ranking of exposure measurement?	
Poor (Uncertain discrimination)	
Adequate	
Good (clearly described, good discrimination)	*

QUALITY 5 – Direct exposure

Ranking of exposure measurement?	
No direct measurement	
Indoor pollution	*
Personal child exposure	**

PART C: Outcome Assessment

QUALITY 6 – PNEUMONIA ASSESSMENT

Ranking of outcome assessment?	
Parent recall	
Fieldworker assessed	*
Physician assessed	**
Radiological	***

QUALITY 7 – BIAS IN ASCERTAINMENT

Is there evidence of ascertainment bias?	
Yes	
Possible	
No	*
If Yes/possible, provide details:	

PART D: Analysis of Results

QUALITY 8 – ADJUSTMENT FOR CONFOUNDING (Balance of randomisation)

Are confounders balanced/ Is there adjustment for confounding?	
No	Confounders not assessed (for randomisation success) or adjusted for if not balanced.
Yes - adequate	The main confounders balanced/ adjusted for (*)

Yes - good	Majority of known confounders balanced/ adjusted for in model (**)

PART E: Qualifying comments

QUALITY 9: indicate overall assessment and specific issues that you would like to draw attention to:

Total Stars	/13 (14 if randomised design = 2 STARS)

STUDY ID		EXTRACTED BY		EXTRACTION DATE	DD	MM	YY

SHORT FORM: METHODOLOGICAL QUALITY APPRAISAL

Exposure to Indoor Air Pollution and adult ALRI: A systematic review and meta-analysis

CASE-CONTROL STUDIES

	WEAK	MODERATE	STRONG	REASON & IMPLICATION
SELECTION PROCEDURES				
EXPOSURE ASSESSMENT				
OUTCOME ASSESSMENT				
CONFOUNDING				