The association between benzodiazepines and asthma exacerbation, influenza-like illness related pneumonia and mortality:

Population-based studies using the United Kingdom Primary care data

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Abstract

Influenza-like illness (ILI) and asthma are common diagnoses in the general practice and exert considerable morbidity and mortality worldwide. One potentially important strategy to reduce this is to determine whether commonly prescribed drugs modify the occurrence and outcomes of respiratory diseases including mortality. The benzodiazepines, a class of psychoactive drugs generally used in the treatment of anxiety and sleep disorders, have recently been suggested as having detrimental effects on immune response to infection, predisposing its users to increased risk of infection and mortality. These drugs have also been implicated in the pathogenesis of asthma. This thesis therefore aimed to investigate whether benzodiazepines modify the occurrence of asthma exacerbation and subsequent mortality in patients with asthma as well as ILI-related pneumonia and ILI-related mortality in patients with ILI.

The Clinical Practice Research Datalink (CPRD) was used as the data source. CPRD contains the medical records of over 13 million patients prospectively collected from over 600 general practices across the United Kingdom and has linkages to the Hospital Episode Statistics database [HES] and national death registry data (the Office of National Statistics [ONS]) which were utilised in this study. CPRD-HES linked data was used to validate diagnoses of asthma exacerbation whereas ONS mortality data validated deaths identified from CPRD. Case-control and cohort study designs were used to investigate associations between benzodiazepines

and the occurrence of asthma exacerbation, ILI-related pneumonia and mortality.

After adjusting for a wide range of potential confounders including physical and psychiatric comorbidities, and concurrent use of other drugs, exposure to benzodiazepines was associated with statistically significant increased occurrence of asthma exacerbation, ILI-related pneumonia and mortality. These associations were observed with both short term and chronic benzodiazepines use. However, the effect of individual benzodiazepines varied across the outcomes of interest with some of the associations lacking statistical significance. For instance, current use of diazepam and temazepam but not lorazepam showed statistically significant associations with increased occurrence of asthma exacerbation.

Overall, findings of this research signal an adverse benzodiazepine effect and hence suggest that a precautionary approach should be exercised when prescribing benzodiazepines in the interim before conclusive evidence is yielded by further research, especially in patients who may already be at increased risk of asthma exacerbation or pneumonia and mortality.

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Table of Contents

Abstract **2**
Acknowledgments **4**
Table of Contents **5**
List of Tables **10**
List of Figures **12**
List of Abbreviations **13**
Chapter 1 : Introduction **15**
1.1 Introduction to thesis and aims 15
1.2 Introduction to asthma 16
1.2.1 Definition and diagnosis of asthma 16
1.2.2 Epidemiology of asthma 17
1.2.3 Pathophysiology and pathogenesis of asthma 18
1.2.4 Asthma management 22
1.2.5 Prognosis in asthma 23
1.3. Introduction to influenza/influenza like illness 24
1.3.1. Definition and diagnosis 24
1.3.2 Epidemiology 27
1.3.3. Pathogenesis and pathophysiology 28
1.3.4. Management 30
1.3.5. Risk factors 31
1.4. Introduction to benzodiazepines 32
1.4.1. Indications for benzodiazepines 32
1.4.2. Benzodiazepines mechanism of action 34
1.4.3. Adverse effects of benzodiazepines 35
1.4.4. Non-benzodiazepine drugs acting on benzodiazepine receptors 37
1.5. Summary of the research questions 38
Chapter 2 : Literature review **40**
2.1 Benzodiazepines and the immune system 41
2.2 Benzodiazepines/Z-drugs and infection 42
2.3 Benzodiazepines/Z-drugs and pneumonia risk 46
2.4 Benzodiazepines and mortality risk 51

Chapter 3 : General methods 58
3.1. Introduction to this chapter 58
3.2. The Clinical Practice Research Datalink 58
3.2.1. History of CPRD 59
3.2.2. Data collection and quality control 59
3.3. CPRD-linked data 62
3.3.1 Hospital Episodes Statistics data 62
3.3.2 Office of National Statistics (ONS) mortality data 63
3.4. Ethics and Data extraction for the PhD research 64
3.5 Data processing 66
3.6. Key study variables 66
3.6.1. Primary outcome variables 66
3.6.2. Exposure variables 68
3.6.3. Covariates 69
3.7. Overview of study designs 73
3.7.1 Asthma exacerbation case-control study 73
3.7.2. Mortality subsequent to asthma exacerbation study 74
3.7.3. Mortality in patients without asthma study 75
3.7.4 ILI-related pneumonia study 76
3.7.5 ILI-related mortality study 76
3.8. Statistical analyses 76
Chapter 4 : Association between benzodiazepine use and exacerbations and mortality in patients with asthma: A matched case-control and survival analysis 78
4.1 Introduction to this study 78
4.2 Background 78
4.3 Methods 80
4.3.1 Data source 80
4.3.2 Sampling frame 80
4.3.3 Outcome definition 80
4.3.4 Exposure definition 82
4.3.5 Potential confounders 84
4.3.6 Statistical analyses 84
4.4 Results 86

4.4.1 Characteristics of study participants 86
4.4.2 Covariate selection 88
4.4.3 Benzodiazepines and occurrence of asthma exacerbation 91
4.4.4 Benzodiazepines and mortality following asthma exacerbation 94
4.4.5 Sensitivity Analyses 99
4.5 Discussion 101
4.5.1 Summary of main findings 101
4.5.2 Strengths and limitations 101
4.5.3 Comparison with existing literature 104
4.5.4 Summary 106
Chapter 5 : The impact of benzodiazepines on all-cause mortality in non-asthma patients: A survival analysis 107
5.1 Introduction to this study 107
5.2 Background 108
5.3 Methods 110
5.3.1 Data source 110
5.3.2 Study design and population 110
5.3.3 Outcome definition 111
5.3.4 Exposure definition 111
5.3.5 Study covariates 112
5.3.6 Statistical analyses 113
5.4 Results 113
5.4.1 Proportional hazards assumption 115
5.4.2 Kaplan Meier Survival curves 118
5.4.3 All-cause mortality 120
5.4.4 Sensitivity analysis 121
5.5 Discussion 122
5.5.1 Summary of main findings 122
5.5.2 Strengths and limitations 122
5.5.3 Comparison with existing literature 123
5.5.4 Summary 125
Chapter 6 : The association between benzodiazepines and influenza-related outcomes (pneumonia and mortality): A survival analysis 126
6.1 Introduction to this chapter 126

6.2 Background 126
6.3 Methods 127
6.3.1 Data source 127
6.3.2 Study design and population 128
6.3.3 Outcome definition 129
6.3.4 Exposure definition 129
6.3.5 Study covariates 130
6.3.6 Statistical analyses 131
6.4 Results 132
6.4.1 Selection of covariates 134
6.4.2 Proportional hazards assumption 139
6.4.3 Benzodiazepines/zopiclone and ILI-related pneumonia 141
6.4.4 Benzodiazepines/zopiclone and ILI-related mortality 144
6.4.5 Sensitivity analyses 146
6.5 Discussion 148
6.5.1 Summary of main findings 148
6.5.2 Strengths and limitations 148
6.5.3 Comparison with existing literature 150
6.5.4 Summary 151
Chapter 7 : Discussion of thesis findings, conclusion and recommendations 153
7.1 Introduction to this chapter 153
7.2 Summary of main thesis findings 154
7.3 Overall discussion of thesis findings 158
7.4 Conclusion, recommendations for practice and future research 167
References 171
Appendices 185
Appendix 1.0: Literature review search strategy in Medline database 185
Appendix 2.0: Benzodiazepines and pneumonia risk: a systematic review and meta-analysis of literature 186
Appendix 3.0: Search terms based on Readcodes for identification of the main study diseases (asthma, ILI and pneumonia). 195
Appendix 4.0: Search terms based on therapeutic codes for identification of study drugs (benzodiazepines and zopiclone) 200

Appendix 5.0: Flow charts summarising the selection strategy for study subjects 208

Appendix 6.0: Asthma study publication 211

Appendix 7.0: Sample size calculations 212

Appendix 8.0: Reviewers comments on the asthma study manuscript submitted to Lancet Respiratory Medicine Journal that led to the non-asthma study 213

Appendix 9.0: Third year annual review: Approval of the five PhD studies by internal examiner 221

List of Tables

Table 1.1: Elimination half-life for benzodiazepines 33

Table 2.1: Studies of benzodiazepines, Z-drugs and infection 44

Table 2.2: Studies investigating the association between benzodiazepines and pneumonia risk 48

Table 2.3: Summary of studies investigating benzodiazepine use and mortality 53

Table 3.1: CPRD data structure 61

Table 3.2: Frequency of asthma Read codes in the asthma study analysis sample 65

Table 3.3: Frequency of ILI Read codes in the ILI study analysis sample 66

Table 3.4: Frequency of Read codes for pneumonia included in the ILI-study analysis sample 68

Table 3.5: Main drug exposure of interest 69

Table 4.1: Characteristics of asthma patients with and without asthma exacerbation (n=131,642) 86

Table 4.2: Association between potential confounders and exposure to benzodiazepines (n=131,642) 89

Table 4.3: Association between benzodiazepines and asthma exacerbation adjusted for individual potential confounders 90

Table 4.4: Association of benzodiazepines/zopiclone exposure with asthma exacerbation (n=131,642) 92

Table 4.5: Association between benzodiazepines and asthma exacerbation stratified by Charlson's comorbidity score categories 93
Table 4.6: Global test for proportional Hazards assumption 95
Table 4.7: Association between benzodiazepines/zopiclone use and all-cause mortality following asthma exacerbation in all ages (n=25,887) . 99
Table 5.1: Characteristics of exposed (benzodiazepine users) and unexposed (benzodiazepine non-users) subjects (n=202,422) 114
Table 5.2: Global test for proportional Hazards assumption 116
Table 5.3: Mortality hazard ratios assessed for the period before and after two-years of follow-up 119
Table 5.4: Association between benzodiazepines/zopiclone use and all-cause mortality among non-asthmatics in all ages (median follow-up period of 7 years, IQR 4.10-7 years [n= 202,422]) 120
Table 6.1: Characteristics of exposed (benzodiazepine users) and unexposed (benzodiazepine non-users) patients (n=804,051) 132
Table 6.2: Association between potential confounders and outcomes of interest (ILI-related pneumonia and mortality [n=804,051]) 136
Table 6.3: Association between benzodiazepines and study outcomes adjusted for individual confounders 139
Table 6.4: Global test for proportional Hazards assumption 141
Table 6.5: Association between benzodiazepines/zopiclone use and ILI-related pneumonia in all ages (n= 804,051) 143
Table 6.6: Association between benzodiazepines/zopiclone use and ILI-related mortality in all ages (n= 804,051) 145

List of Figures

Figure 1.1. The interplay between airway inflammation, pathophysiological changes in the airway and asthma clinical symptoms. 21

Figure 1.2: Diagnostic assays available in different laboratory settings. 26

Figure 1.3: Benzodiazepine-type anxiolytics: Average national consumption, by region, 2004-2012¥† 33

Figure 4.1: Log-log plots examining the Cox proportional hazards assumption 95

Figure 4.2: Kaplan Meier survival curves for study drug exposures (over a median follow-up period of 2 years) 97

Figure 5.1: Log-log plots examining Cox proportional hazards assumption 116

Figure 5.2: Kaplan Meier survival curves for study drug exposures (over a median follow-up of 7 years) 118

Figure 6.1: Log-log plots examining Cox proportional hazards assumption for the association between drug use and ILI-related pneumonia 140

Figure 6.2: Log-log plots examining Cox proportional hazards assumption for the association between drug use and ILI-related death 140

Figure 6.3: Adjusted survival curves for the association between study drugs and ILI-related pneumonia 142

Figure 6.4: Adjusted survival curves for the association between study drugs and ILI-related mortality 144

Figure 7.1: Summary of the overall thesis findings 157

List of Abbreviations

CPRD	Clinical Practice Research Datalink
GPRD	General Practice Research Database
HES	Hospital Episode Statistics
ONS	Office of National statistics
GABA	Gamma amino butyric acid
BZPs	Benzodiazepines
BNF	British National Formulary
CAP	Community acquired pneumonia
ILI	Influenza-like illness
RIDT	Rabid Influenza Diagnostic Test
CI	Confidence Interval
OR	Odds ratio
HR	Hazard ratio
IQR	Interquartile range
NNH	Number Needed to Harm
AF	Attributable fraction
PAF	Population attributable fraction
COPD	Chronic obstructive pulmonary disease
ICD	International Classification of Diseases
UTS	Up to Standard
NHS	National Health Service
UK	United Kingdom
WHO	World Health Organisation
MHRA	Medicines and Health products Regulatory Agency

BMI	Body mass index
IMD	Index of Multiple Deprivation
PH	Proportional hazards
KM	Kaplan Meier
LABA	Long-acting beta-2-agonists
SABA	Short-acting beta-2-agonists

Chapter 1 : Introduction

1.1 Introduction to thesis and aims

Influenza-like illness (ILI) and asthma cause considerable morbidity and mortality in the United Kingdom. One potentially important strategy to reduce this is to determine whether commonly prescribed drugs modify the outcomes of respiratory diseases and, where choices are available, modify national guidelines and identify alternate therapies on prescribing accordingly.

Recent evidence has suggested an association between benzodiazepines and increased susceptibility to infection and mortality. Benzodiazepines have also been implicated in the pathogenesis of asthma. The purpose of this thesis was therefore to investigate the association between benzodiazepines and the occurrence of asthma exacerbation, ILI-related pneumonia and mortality using the Clinical Practice Research Datalink (CPRD). Specific objectives were as follows:

1) To investigate the association between benzodiazepines and exacerbation of asthma in patients with asthma

2) To investigate the association between benzodiazepines and mortality subsequent to asthma exacerbation in patients with asthma

3) To investigate the association between benzodiazepines and ILI-related pneumonia in patients with ILI

4) To investigate the association between benzodiazepines and ILI-related mortality in patients with ILI

A post hoc study was added to this thesis in consultation with the doctoral supervision team following peer reviewer recommendations for the asthma study manuscript submitted to the Lancet Respiratory Medicine Journal. The post hoc study investigated the association between benzodiazepines and mortality among non-asthma patients.

In sections 1.2-1.5 below, brief introductions to asthma, ILI and benzodiazepines are presented. The literature review and general study methodology follow in chapter 2 and 3 respectively. The subsequent chapters 4-6 address the individual study objectives and finally, chapter 7 presents an overarching discussion, conclusions and recommendations for practice and future research.

1.2 Introduction to asthma

1.2.1 Definition and diagnosis of asthma

Asthma is a respiratory disease commonly characterised by variable and recurring symptoms, bronchial hyper-responsiveness, airflow obstruction and an underlying airway inflammation.[1] Clinical symptoms of asthma include breathlessness, wheeze, cough and chest tightness which are particularly worse at night and early in the morning.[2]

Unlike many other diseases for which confirmatory diagnostic tests are present, in the diagnosis of asthma there are no definitive examinations or laboratory findings.[3] The diagnosis of asthma is mostly a clinical one,

based on identifying both a characteristic pattern of asthma symptoms and variable expiratory airflow limitation.[4, 5] The pattern of respiratory symptoms is particularly important as the symptoms may be due to respiratory disease other than asthma.[5]

1.2.2 Epidemiology of asthma

Asthma is one of the most common chronic conditions affecting both children and adults. For the past four decades, there has been an increase in the prevalence of asthma in most countries with current global estimates of 235 million people suffering from asthma[6] and an additional 100 million persons expected by 2025 if the current trend persists.[7] Although the prevalence of asthma in the UK has plateaued since the 1990s, it is still among the highest in the world with an estimated 5.4 million people currently receiving treatment for asthma of which 1.1 million are children.[8]

There is a substantial number of primary care consultations and hospital admissions due to asthma every year; for instance, an average primary care trust in the UK with a registered population of a third of a million can expect to treat 25,000 people with asthma and over 400 asthma patients to be admitted to hospital each year.[9]

Mortality estimates of asthma greatly differ between countries and within countries, ranging from 0 to ≥10.1 per 100, 000 people with asthma.[6] Globally, 250,000 deaths occur annually.[10] According to several sources including the World Health Organisation (WHO), many of these deaths are preventable with proper long-term medical care and patient education

especially relating to medication adherence and the importance of seeking emergency medical help.[6, 7, 11] Indeed, many asthma deaths have been observed in poor countries with limited asthma care, particularly because of the lack of access to essential asthma drugs (inhaled corticosteroids).[6]

Whilst the aetiology of asthma is not fully established, it is widely accepted that the underlying causes of asthma involve a combination of genetic predisposition with various environmental factors[2, 12]that may provoke allergic reactions or irritate the airways, such as tobacco smoke, chemical irritants in the workplace, indoor allergens (for example, house dust mites in bedding, carpets and stuffed furniture and animal dander), outdoor allergens (such as moulds and pollen) and air pollution.[6] Other risk factors for asthma include lower socioeconomic status and the female sex in young adults.[6, 12]

1.2.3 Pathophysiology and pathogenesis of asthma

Asthma is widely accepted as an inflammatory airway disease. Post-mortem studies on patients dying from status asthmaticus (severe asthma attack) in the nineteenth century showed eosinophilic infiltration in bronchial mucosa, increased thickness of smooth muscle in bronchial walls, mucus gland hyperplasia and hypertrophy, occlusion of airways with thick mucus plugs and loss of bronchial epithelium.[3] More recently, various studies using bronchoscopy, induced sputum and broncho-alveolar lavage have also demonstrated evidence in support of airway inflammation, for example increased eosinophils, mast cells, neutrophils (in chronic severe

asthma) and changes in cytokine profile and concentration in patients with asthma including mild asthma.[13]

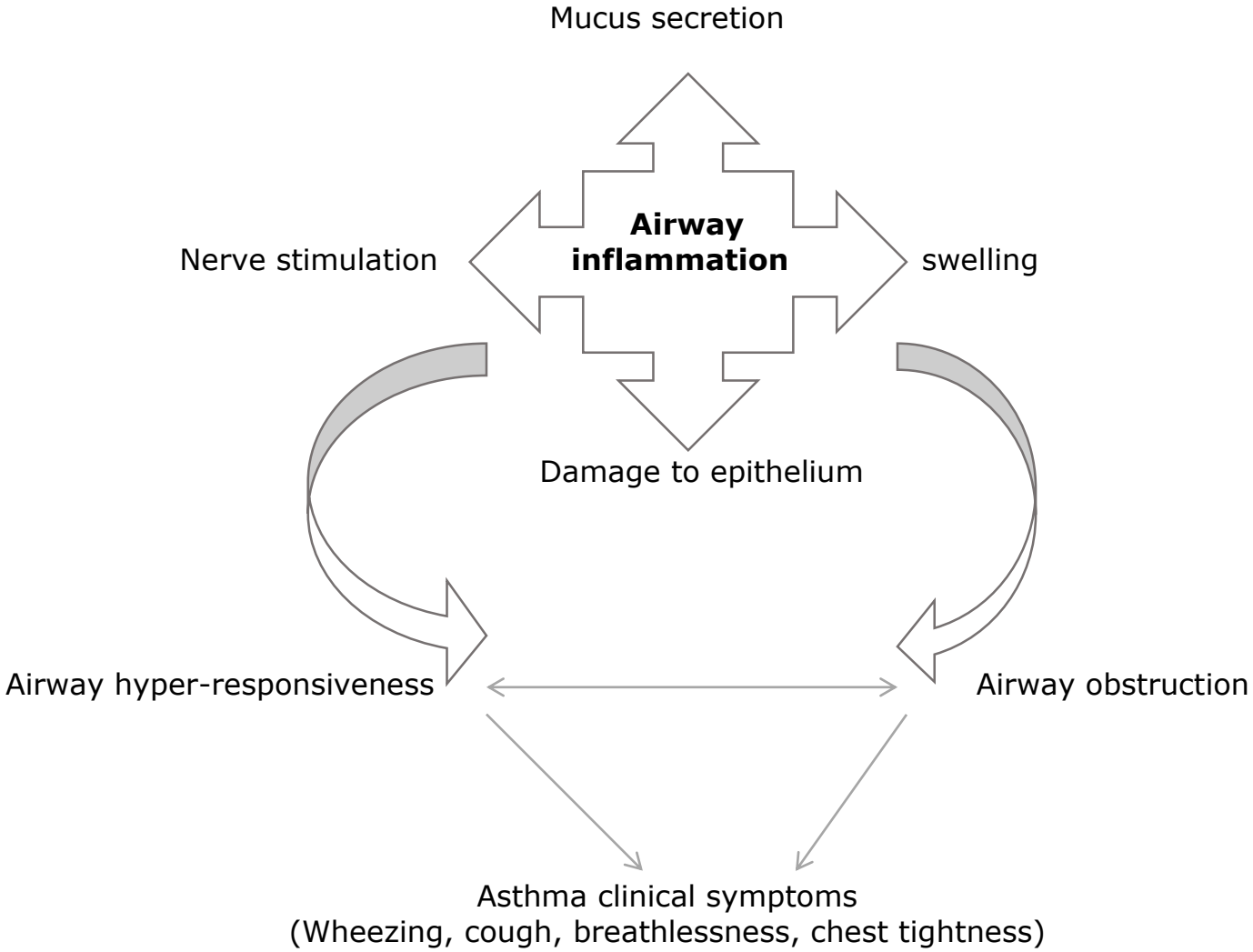
The airway inflammation in asthma involves a coordinated interplay of the respiratory epithelium, innate immune system, and adaptive immunity (which initiates and drives a chronic inflammatory response). Central to this inflammation is an interaction between genes and the environment which results in an abnormal immune response to allergens and other triggers in genetically susceptible individuals.[1, 13]

The inflammatory response in asthma follows excessive activation of mast cells in the airway and their subsequent degranulation which results in the release of bronchoconstrictor mediators and a number of pro-inflammatory factors including histamine, proteases, tissue necrosis factor and leukotrienes that cause an immediate hypersensitivity response and bronchoconstriction (an early asthmatic response).[3] Some of the inflammatory mediators released particularly the leukotrienes, lead to the recruitment of cells involved in inflammation (such as eosinophils, neutrophils and basophils and T-lymphocytes) to the airways. These cells then release other mediators which lead to degranulation of activated inflammatory cells especially eosinophils, culminating in what is termed as the late asthmatic response.[3] This late asthmatic phase is characterised by leucocyte infiltration, epithelial damage and further bronchospasms and occurs between 18 and 24 hours post exposure to an allergen or any other asthmatic trigger such as bacteria or viruses. Over time, the ongoing inflammation results in structural change within the airways (airway

remodelling) including epithelial damage, subepithelial fibrosis, mucus hypersecretion and goblet cell hyperplasia and smooth muscle hypertrophy, which lead to increased airway hyper-responsiveness (exaggerated airway narrowing in response to exogenous and endogenous stimuli) and airflow obstruction (Figure 1.1).[3, 14]

In asthma exacerbation, which is characterised by progressive worsening of asthma symptoms, the inflammation of the airway is intense and not easily controlled by anti-inflammatory drugs.[3] While this phenomenon may be entirely explained by the severity of chronic asthma, the alternative explanation may be the modification of the airway inflammatory phenotype which follows an acute asthma exacerbation trigger.[15]

Figure 1.1. The interplay between airway inflammation, pathophysiological changes in the airway and asthma clinical symptoms.



1.2.4 Asthma management

As asthma is a chronic disease, currently with no cure, the goal of management is to control this condition mainly through control of symptoms, avoidance of asthma triggers (where possible) and minimising future risk (of exacerbations, fixed airflow limitation and side-effects of treatment).[5, 16]

The mainstay of asthma management involves multiple drug therapies categorised as controller (preventer) and reliever (rescue) medication mostly administered to the patient following a 'step-wise' approach whereby the patient is started at the step most appropriate to the degree of asthma severity and increased or decreased as necessary to achieve and maintain adequate control.[4] The controller medications are used by the patient over the long term to control persistent asthma. These include anti-inflammatory agents such as corticosteroids, leukotriene modifiers and long acting bronchodilators such as long-acting beta-2-agonists (LABA). Reliever medications on the other hand, are for rapidly reversing bronchoconstriction and associated symptoms and as such are provided to all patients to use as-needed. They include short acting beta-2-agonists (SABA), short-acting anti-cholinergic agents and short acting theophylline.[2, 4]

There is a third category of asthma drugs called 'add-on therapies' for patients with severe asthma which are considered when patients have persistent symptoms and/or exacerbations despite optimised

treatment with high dose controller medications (usually a high dose of inhaled corticosteroids and a long-acting beta-2-agonists) and for treatment of modifiable risk factors.[2]

1.2.5 Prognosis in asthma

It is estimated that in 90-95% of asthma patients control of asthma symptoms and normalisation of lung function can be achieved,[17] indicating that the majority of asthma patients can live a normal and active life.[16] Prognosis in asthma largely depends on the type of asthma with early onset of asthma (<16 years) and intermittent asthma largely associated with a better prognosis.[18] Factors predicting poor prognosis in asthma patients include:

- Chronic or acute bronchitis
- Late-onset asthma
- Underlying comorbidity including hypertension, diabetes and heart failure, ischaemic heart disease and cor pulmonale
- Age over 75 years
- Non-adherence to treatment.[2, 18, 19]

1.3. Introduction to influenza/influenza like illness

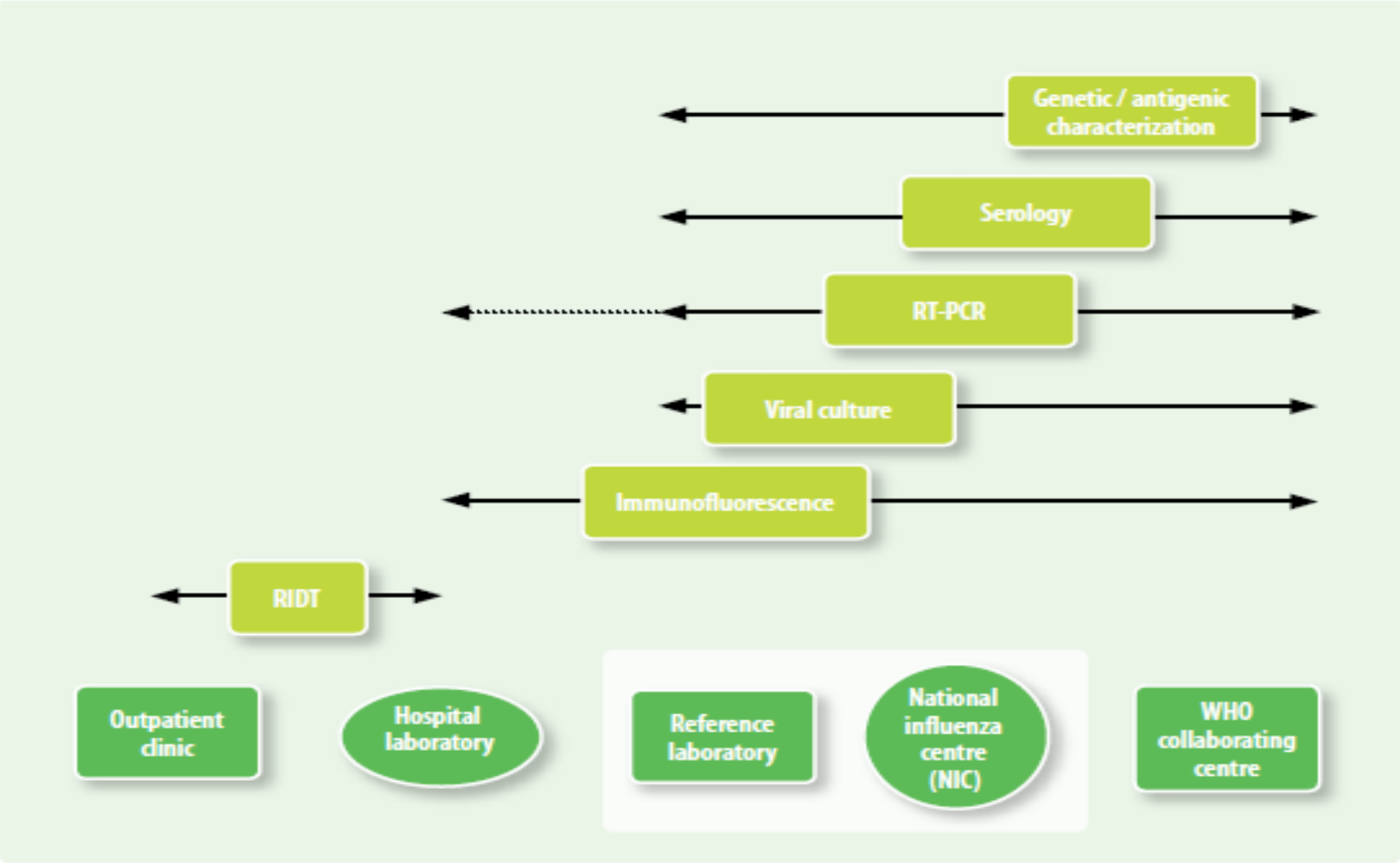
1.3.1. Definition and diagnosis

Influenza is an acute respiratory infection caused by influenza viruses that are of three types: A, B and C, with influenza A and B causing most sickness in humans. Clinical symptoms of influenza may include fever, chills, headache, myalgia and extreme fatigue, dry cough, sore throat and stuffy nose.[20, 21] However, it is not only influenza infection that is characterised by these symptoms; other respiratory viral infections cause a similar clinical picture. This has led to the use of the term influenza-like illness (ILI) to describe the clinical syndrome that may be attributed to influenza and other common respiratory viral pathogens (e.g. respiratory syncytial virus, rhinovirus, parainfluenza virus, adenovirus and human metapneumovirus).[22] Each of these pathogens has been shown to cause ILI and has the potential of becoming an independent health threat (for example, cause severe disease and complicate underlying disease).[23]

A clinical diagnosis of influenza may be made based on signs and symptoms especially in outpatient settings like general practice. However, as influenza may be clinically indistinguishable from other influenza like illnesses, laboratory tests are usually required to reach a definitive influenza diagnosis.[21] These diagnostic tests include: rapid influenza diagnostic tests (RIDT), viral culture, serology, polymerase chain reaction (PCR), rapid molecular assays) and

immunofluorescence assays.[24, 25] With the exception of RIDT which can easily be performed at the point of care and in remote settings, laboratory diagnosis of influenza requires highly skilled laboratory staff and sophisticated laboratory capacity (Figure 1.2).[24] In addition, the use of RIDT is associated with low sensitivity estimated at 50-70% in comparison to viral culture or reverse transcription polymerase chain reaction (RT-PCR) hence a high possibility of false negative test results.[24, 25] Thus, the diagnosis of influenza is usually a clinical one and has been found to be reasonably reliable especially when combined with information on circulating viruses in the community.[21, 22, 26]

Figure 1.2: Diagnostic assays available in different laboratory settings.



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1.3.2 Epidemiology

Although influenza viruses may be identified at other times of the year, annual outbreaks usually intensify during winter in temperate regions.[27] There is less seasonality associated with influenza virus circulation in tropical climates.[21, 22] The assessment of the impact attributable to influenza outbreaks is not straightforward due to factors like the non-specific nature of influenza illness and secondary infections which can lead to inaccurate hospital records and death certification.[28] As such indirect measures, for instance consultation rates for ILI in general practices (GP)[29] and excess mortality (deaths above the expected seasonal level)[20] have been used to assess the impact of influenza outbreaks.

In the UK, annually, 800,000-1,164,000 GP consultations, 19,000-31,200 hospital admissions and 18,500-25,000 deaths were attributed to influenza using data from 1996 to 2004.[30] Such estimates are comparable to those from other countries for instance in the United States (US), the Centre for Disease Control (CDC) estimates that influenza associated deaths ranged from 3,000 to 49,000 between 1976 and 2007.[31] The impact of influenza can be enormous during pandemics, with the classic example of the 1918-19 influenza pandemic that is believed to have affected 25% of the world‘s population and led to the death of at least 50 million people.[32]

Most deaths especially during seasonal influenza occur in the elderly,[33] while children, particularly those under six years, experience the highest attack rates and hospitalisation.[27] Hospitalisations and deaths from influenza complications are also increased among those in 'clinical risk groups' which include chronic heart disease, chronic respiratory disease, chronic liver disease and immunosuppression.[20, 21, 27]

The influenza season is also heavily characterised by absenteeism from school and work which contributes to the family and country's economic burden in terms of lost productivity from missed work days. For example in the United States, the loss of earnings due to influenza is projected at $16.3 billion per year.[34]

1.3.3. Pathogenesis and pathophysiology

Influenza/ILI viruses may enter the respiratory tract via a number of routes including direct inhalation of infectious respiratory droplets.[35] After entry the viruses attach to and replicate in respiratory epithelial cells, located on the outer surface of most of the airway, followed by the release of progeny virus particles (copies of replicated virus) from an infected cell and cell death.[36] The virus surface proteins known as Heamagglutinin (HA) and neuraminidase (NA) play important roles in the pathogenesis of influenza with HA facilitating viral binding onto respiratory epithelial surface receptors and entry into cells while NAs

are involved in the release of progeny virus particles from an infected cell.[21]

Both innate and adaptive immune responses are involved in an influenza/ILI infection. An initial innate response is characterised by cytokine release (IFNα/β), influx of natural killer cells or neutrophil granulocytes and cell activation, and is responsible for the rapid onset of ILI clinical symptoms.[21] It is likely that the damaging effects of the infection on respiratory epithelium and the localised inflammation account for respiratory tract symptoms while systemic symptoms such as myalgia and fever are caused by the release of inflammatory mediators such as cytokines.[37]

The adaptive immune response is associated with development of strain-specific antibodies which offer protection against reinfection by the same virus species. However, the influenza virus has the potential to evade destruction by antibodies because of variability in its structural proteins in the form of antigenic drift (slight structural variations) or shift (major change in surface proteins).[21] This has led to annual updates of influenza vaccines to capture circulating influenza viral strains.

Influenza is usually complicated with bacterial superinfection, predominantly streptococcus pneumoniae, due to a number of factors including increased bacterial cell adherence to epithelial lining and tissue invasion because of damage to respiratory epithelium and

impaired ciliary clearance, decreased polymorphonuclear and alveolar macrophage chemotaxis and suppression of other host immune responses caused by influenza virus infection.[21, 38]

1.3.4. Management

Uncomplicated influenza is treated symptomatically, especially in adolescents and young adults.[21] Currently two classes of antiviral drugs are available for the management of influenza namely Neuraminidase inhibitors (e.g. oseltamivir and zanamivir) and inhibitors of viral M2 protein (such as amantadine and rimantadine).

According to the UK's guidelines for treatment of influenza[39], previously healthy adults and children with uncomplicated influenza do not require treatment with antivirals unless complications are likely to develop as judged by the physician. Antivirals are recommended for use in at risk population groups including pregnant women and in all patients with complicated influenza. Antiviral drugs are also used as post exposure prophylaxis for influenza, for example, oseltamivir may be given to carers of influenza patients.[39] Strategies are also put in place to prevent infection through annual influenza vaccination.[20]

1.3.5. Risk factors

Influenza affects all people of any age group. However, the risk of complications is increased in certain conditions including:[20]

a. Neurological, hepatic, renal, pulmonary and chronic cardiac disease

b. Diabetes mellitus

c. Immunosuppression

d. Age over 65 years

e. Pregnancy (including up to two weeks post-partum)

f. Morbid obesity (BMI >=40).

1.4. Introduction to benzodiazepines

1.4.1. Indications for benzodiazepines

Benzodiazepines are one of the WHO essential medications[40] widely used in the treatment of various health disorders. They are widely prescribed especially in the treatment of insomnia and anxiety.[41] It is currently estimated that about 10-20% of the global population use sleeping pills or tranquilizers to relieve insomnia and other sleep disorders.[42] There are regional variations in the use of benzodiazepines as anxiolytics (to relieve anxiety) with Europe shown to have the highest average national consumption rates (using standardised defined daily doses for statistical purpose[S-DDD]) for the period between 2004 and 2012 (Figure 1.3).[43]

Individual benzodiazepines work in a similar manner to each other as most of them mainly used as anxiolytics will induce sleep and most of those classified as hypnotics also have a sedative action.[44] However, different benzodiazepines vary in the rate at which they are metabolised to pharmacologically active forms and particularly in their half-lives (Table 1.1), which may explain the differences in their clinical effects and associated adverse events.[44]

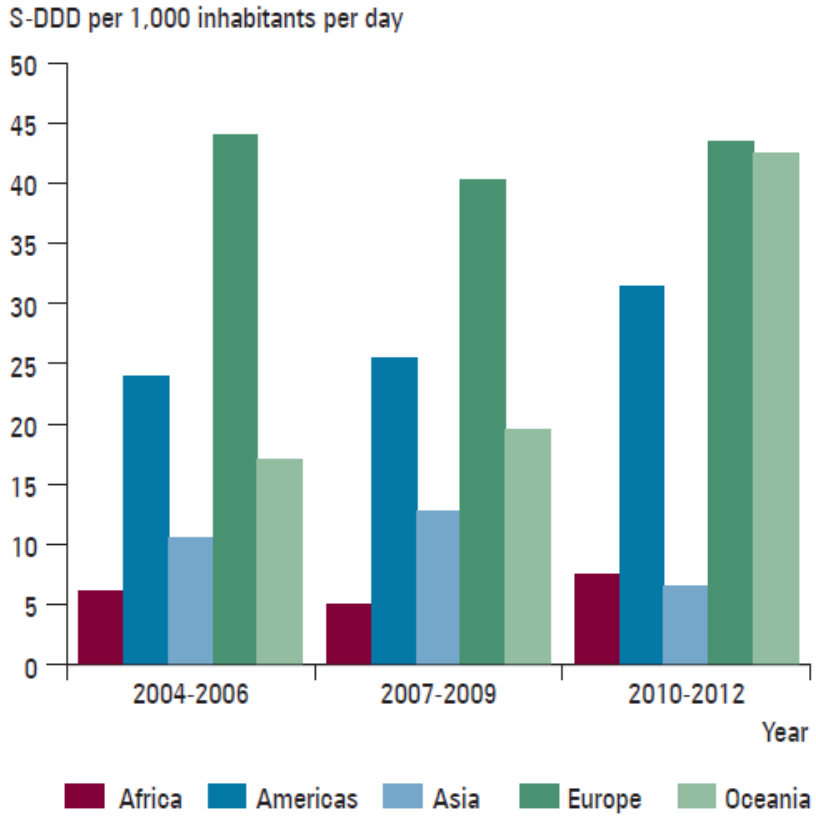
These drugs are also widely prescribed for other reasons, such as convulsive disorders (e.g. epilepsy), muscle spasticity, pre-surgical sedation, detoxification from alcohol and other substances, involuntary movement disorders, palliative care and anxiety

associated with cardiovascular or gastrointestinal or respiratory conditions.[41, 44, 45]

Table 1.1: Elimination half-life for benzodiazepines

Benzodiazepine class	Example	Half-life (hours)[41, 44]
Short acting	Midazolam	2.5
	Triazolam	3-4
Intermediate acting	Alprazolam	12-15
	Lorazepam	12-16
	Oxazepam	7-20
	Temazepam	7-11
Long acting	Chlordiazepoxide	30
	Diazepam	25-100
	Clonazepam	18-50
	Flurazepam	25-1000

Figure 1.3: Benzodiazepine-type anxiolytics: Average national consumption, by region, 2004-2012¥†



¥Statistical data submitted by Governments are used to calculate the average annual consumption for a three-year period

S-DDD denotes Defined Daily Doses for statistical purpose

† Figure reproduced with copyright permission.[43]

1.4.2. Benzodiazepines mechanism of action

Benzodiazepines (BZPs) are a class of psychoactive drugs which enhance the functioning of γ-amino-butyric acid (GABA), a principal inhibitory neurotransmitter in the central nervous system (CNS).[46] BZPs target GABAA receptors,[47] the binding site for GABA, acting allosterically (i.e. binding to a site on the GABAA receptor different from that of the neurotransmitter GABA) to modulate the chloride ion channel opening frequency.[48, 49] The GABAA receptor comprises five protein subunits that surround a central ion channel crossing the cell membrane, which conducts chloride and other anions.[50] The majority of GABAA receptor subunits are α, β, and γ. The most prevalent GABAA receptor in the brain is composed of α1, β2, γ2 subunits with β subunit for the binding of GABAAnd the interface between the α1 and γ2 subunits conferring sensitivity to benzodiazepines (thus referred to as benzodiazepine receptor).[48, 50]

When the neurotransmitter GABA binds with the GABAA receptor, a conformational change in the receptor is triggered, allowing more chloride to enter the cell.[48] This results in hyperpolarisation of the cell membrane, leading to reduced neuronal excitability and a general

inhibitory effect of neuronal activity.[48] The allosteric binding of BZPs locks the GABAA receptor into a conformation that enhances the binding of GABA resulting in an amplified inhibitory effect.[51]

Benzodiazepine receptors in the CNS are responsible for most of the clinical benefits and side effects of BZPs.[52] There is an emerging field of study for additional actions of BZPs, mediated by benzodiazepine receptors situated in other parts of the body including the heart, salivary glands, kidney, lung, the nasal and lingual epithelia, the lining of the pulmonary arteries, the thymus, and the bone marrow.[53, 54]Activation of these receptors has been associated with diverse actions such as steroidogenesis,[55] cellular proliferation,[56] apoptosis[57] and immunomodulation.[58]

A recent study by Sanders et al[58] showed the influence of GABA on the innate immune responses to infection as attributable to the acidification of the cytoplasm of immune cells especially macrophages and monocytes. In their study, augmenting GABA signalling with diazepam increased intracellular H+ concentration leading to reduced TNF-alpha production and impaired bacterial phagocytosis, and culminating in the increase in infection risk and mortality.[58]

1.4.3. Adverse effects of benzodiazepines

According to the British National Formulary,[41] use of hypnotic drugs should be limited to short courses (2-4 weeks) and should generally be avoided in the elderly due to the associated adverse effects

especially with long term use. Despite this warning, BZPs are known to be prescribed for longer than is recommended and in excessive doses, particularly in older adults.[59, 60]This may partly be due to the quick relief of symptoms BZPs give which is usually followed by physical and psychological dependence and tolerance to these drugs.[41]

As a result of physiologic dependence, withdrawal symptoms, mainly anxiety symptoms[61] may occur especially with abrupt withdrawal of benzodiazepines.[62] In addition, rebound-insomnia, autonomic instability (i.e., increased heart rate and blood pressure level, diaphoresis, tremulousness), sensory hypersensitivity, seizures and delirium tremens are common BZPs withdrawal symptoms.[61]

BZPs are usually contra-indicated in pregnancy and in breastfeeding mothers. These drugs cross the placenta and are also secreted in breast milk and may lead to the development of dependence and consequent withdrawal symptoms in the foetus.[63]

Increased irritability, hostility, aggression, impulsivity and excitement may occur in some patients who take BZPs.[41, 64]This paradoxical disinhibition by BZPs may, in rare cases, result in attacks of violence or other indiscretionary or antisocial behaviours.[64]

BZPs also induce anterograde amnesia, with episodic memory (the remembering of recent events and the circumstances in which they occurred and their time sequences) being particularly impaired and

more markedly so in heavy alcohol drinkers who also use benzodiazepines.[62, 65]

BZPs have also been associated with psychomotor retardation occurring with initial administration of the drug or with a sudden therapeutic dosage increase.[62] This may be more pronounced in the elderly or patients with comorbid illnesses who have decreased rates of metabolism or greater susceptibility to CNS depression.[66] Psychomotor symptoms include poor concentration, drowsiness, ataxia, motor incoordination, dysarthria, muscle weakness, diplopia, mental confusion and vertigo.[65] Studies of the psychomotor effects suggest that BZPs slow reaction time and impair driving skills, increasing the risk of motor vehicle accidents in patients who are taking these drugs.[67] BZP use is also associated with increased risk of falls,[68] hip fractures,[69, 70] and cognitive decline[71] including dementia[72] among the elderly.

In addition, these drugs have also been linked to increased risk of infection[73] and mortality as mentioned earlier.[74, 75]Chapter 2 includes a detailed literature review on BZP use and infection risk and mortality.

1.4.4. Non-benzodiazepine drugs acting on benzodiazepine receptors

In the quest for hypnotic drugs that may overcome adverse events associated with BZPs, non-benzodiazepines known as the Z-drugs

(zopiclone, zolpidem and zaleplone) were introduced in the late 1980s and 1990s.[76] Like BZPs, Z-drugs potentiate the functioning of GABA by allosteric binding on γ-aminobutyric acid-type A (GABAA) receptor.[77, 78]There are minor differences between BZPs and Z-drugs that may be related to their pharmacology.[76] Z-drugs generally possess a shorter duration of action and half-life compared to some of the BZPs.[77]

Over the past decade, there has been an increase in prescribing of Z-drugs, partly because these drugs were marketed as safer and less liable to dependence compared with BZPs despite lack of evidence in support of their alleged safety.[79] Several adverse effects have been reported in users of Z-drugs including cognitive problems,[80] psychomotor impairment[81] and depression.[82]

1.5. Summary of the research questions

To recap, the objectives of this thesis were as follows:

1) To investigate the association between benzodiazepines and exacerbation of asthma in patients with asthma
2) To investigate the association between benzodiazepines and mortality subsequent to asthma exacerbation in patients with asthma
3) To investigate the association between benzodiazepines and mortality in patients without asthma

4) To investigate the association between benzodiazepines and ILI-related pneumonia in patients with ILI
5) To investigate the association between benzodiazepines and ILI-related mortality in patients with ILI.

Chapter 2 : Literature review

The main aim of the literature review was to identify studies investigating the impact of benzodiazepines on the outcomes of asthma and influenza-like illness (ILI) i.e. asthma exacerbation, ILI-related pneumonia and mortality subsequent to exacerbation of asthma or ILI. An exhaustive search was undertaken in the Medline database (Search strategy: Appendix 1.0) and modified to suit the search criteria in the Embase, Pubmed and Web of science databases using the following terms and Boolean operators: (benzodiazepines OR diazepam OR lorazepam OR midazolam OR nitrazepam OR alprazolam OR chlordiazepoxide OR valium OR xanax OR lexomil OR ativan OR librium OR Z-drugs OR zopiclone OR zolpidem OR zaleplon OR hypnotics OR Anti-Anxiety Agents OR anxiolytic OR tranquilisers Or sleeping pills) AND (asthma OR influenza OR influenza-like illness OR ILI OR Pneumonia OR respiratory tract infections OR mortality OR death). Additional studies were identified from the reference lists of relevant studies. No restrictions were applied to the search strategy, for example publications of any type (animal and human), language, year and setting were retrieved.

Despite conducting a rigorous search as stated above, there is at present, a paucity of epidemiological studies on influenza/ILI and asthma outcomes with respect to the use of benzodiazepines. However, a reasonable number of studies were found that assessed

the effects of benzodiazepines and related drugs (Z-drugs) on the risk of infection and mortality, providing compelling evidence as a basis for the current studies on influenza/ILI and asthma outcomes presented in Chapters 4 to 6 of this thesis. Relevant studies from published literature are discussed below, categorised as studies of benzodiazepines and the immune system, animal and human studies and covering the following three outcomes: infection other than pneumonia, pneumonia and mortality.

2.1 Benzodiazepines and the immune system

A systematic review of available literature suggests that benzodiazepines exhibit anti-inflammatory actions against the innate immune system.[83] Most of the published studies focus on impairment of functions of macrophages and neutrophils by benzodiazepines (mainly midazolam and diazepam). Midazolam has been shown to inhibit the expression of pro-inflammatory mediators, namely inducible nitric oxide synthase and cyclooxygenase in the lipopolysaccharide (LPS)-activated macrophages.[84] Furthermore, midazolam suppressed LPS-induced TNF-α activity in macrophages (leading to dysregulation of cytokine production), an effect that was blocked by the peripheral benzodiazepine receptor antagonist PK 11195.[85] In both neutrophils and macrophages, impairment of oxidative burst and phagocytosis, which weakens the immune

response to infection, has been reported with benzodiazepine treatment in animal studies.[58, 86, 87]

The evidence of these drugs' effects on the adaptive immune system is still developing with data from a study in mice suggesting impairment of humoral responses following long-term (60 days or greater) administration of diazepam in sub-sedative doses.[88]

2.2 Benzodiazepines/Z-drugs and infection

The impact of benzodiazepines on infection and mortality is a relatively new study area with much of the evidence established within the two last decades.

In animal studies, benzodiazepines increased mortality from Klebsiella pneumoniae,[74] Mycobacterium bovis,[89] Salmonella typhimurium,[88] Influenza and Streptococcus pneumonia[58] infections (Table 2.1). In the latter study, Sanders et al. also studied the impact of diazepam on mortality in mice made vulnerable to streptococcus pneumoniae by prior influenza infection, a scenario that mimics a common occurrence in human influenza infection, and found an increased risk of death with diazepam treatment (HR 22.4; 95% CI 4.3-117.5).[58] Similarly, in critical care patients sedation with the benzodiazepine midazolam was associated with a doubling of secondary infection compared to dexmedetomidine (19.7 vs. 10.2%; $p = 0.02$).[90] A meta-analysis of evidence from randomised controlled trials of commonly prescribed Z-drugs (zopiclone, zolpidem,

Ramelteon and Zaleplon) found increased risk of infection with Z-drugs compared to placebo (risk ratio 1.44; 95% CI 1.25-1.64).[91]

On the other hand, a randomised controlled trial of pre-operative diazepam use on surgical site infection revealed a protective effect of diazepam against infection. An overall lower incidence of surgical site infection, within 30 days of follow-up, was found with diazepam treatment (placebo vs diazepam treated relative risk 1.79; 95% CI 1.31-2.43).[92] However, the diazepam-protective effect lost significance in subgroup analyses that took account of anxiety (placebo vs diazepam treated relative risk in mild anxiety 1.56; 95% CI 0.93-2.67).[92] The inconsistency in the findings of this study suggests other possible non-causal mechanisms underlying the previously observed protective effect of benzodiazepines and infection risk (confounding). Table 2.1 summarises studies of benzodiazepines/Z-drugs and infection.

Table 2.1: Studies of benzodiazepines, Z-drugs and infection

Author/year Country	Study type	Study design	Drug	Type of infection	Brief methods	Results
						Deleterious effects
Lewis SC et al 2014 USA	Human	Case -control	Benzodiazepines (as a class of drugs)	Unable to ascertain	110 patients with ventilator-associated conditions matched to 110 controls on the basis of age, sex, ICU type, comorbidities, and duration of mechanical ventilation prior to ventilator-associated conditions.	Starting benzodiazepines prior to intubation was associated with infection-related ventilator-associated complications (odds ratio, 5.0; 95% CI, 1.3-29)
Sanders RD et al 2013 UK	Animal	Experimental	Diazepam	Streptococcus pneumoniae	Treatment of murine *Streptococcus pneumoniae* pneumonia model with subsedative anxiolytic doses of diazepam (i.p 2mg/kg).	Increased mortality and bacterial load from *Streptococcus pneumoniae* pneumonia.
Huemer HP et al 2010 Austria	Animal	Experimental	Diazepam	Cowpox and vaccinia virus	Mice treated with diazepam and infected with cowpox and vaccinia virus	Earlier disease onset and prolonged duration of symptoms.
Joya FL et al 2009 USA	Human	Meta-analysis (US Food and Drug Administration data, and other published studies)	Z-drugs (zopiclone, zolpidem, Ramelteon and Zaleplon)	Any kind of infection	8828 participants received Z-drugs and 4383 Participants randomly received placebos.	Higher infection reports among the Z-drug group than placebo group (risk ratio 1.44; 95% CI 1.25-1.64)
Domingues-Junior M et al 2000 Brazil	Animal	Experimental	Diazepam	Mycobacterium bovis	i.p diazepam 6 days prior to mycobacterium bovis (M.bovis) infection in Hamsters	Increased mortality and increased scores of M.bovis colony forming units in lungs, liver and spleen.
Galdiero F et al 1995 Italy	Animal	Experimental	Diazepam	Salmonella typhimurium	Chronic i.p diazepam treatment (60-90 days) in mice	Increased mortality after 90 days of diazepam treatment
Laschi A et al 1983 France	Animal	Experimental	Diazepam	Intraperitoneal (i.P) *Klebsiella pneumonia*	i.p diazepam 3 days before infection in mice	Increased mortality.

Author/year Country	Study type	Study design	Drug	Type of infection	Brief methods	Results
						Protective effects
Dugan AL et al 2010 USA	Animal	Experimental	Midazolam	Pseudomonas aeruginosa	Midazolam (i.p1mg/kg) treatment 2 days pre- Pseudomonas aeruginosa infection and 7 days post-infection of stressed mice.	Decreased mortality.
Levandovski R et al 2008 Brazil	Human	Randomized, double-blinded, placebo-controlled Trial	Diazepam	Any kind of infection	Random assignment of oral diazepam 10mg to 65 patients and placebo to 65 patients a night before and 1 hour prior to undergoing abdominal hysterectomy. A diagnosis of surgical site infection obtained using the criteria of the Centers for Disease Control and Prevention within a standard follow-up period of 30 days.	Occurrence of surgical site infection was increased in placebo-treated patients compared to patients treated with diazepam. Placebo-treated relative risk (RR): RR 1.79; 95% CI 1.31-2.43. Sub group analysis according to level of anxiety showed a reduction in the RR to SSI: -highly anxious patients: RR 1.65; 95% CI 1.07-2.56 -mildly anxious patients: RR 1.56; 95% CI 0.93-2.67
Farrington M et al 1994 UK	Laboratory (in vitro)	Experimental	Midazolam	Staphylococcus epidermidis	Culture of residual infusion fluids from 50 midazolam infusion syringes. Infusions were prepared under aseptic precautions and run for between 0.75 and 21.25 hours	Scanty growths of Staphylococcus epidermidis detected from 4 syringes. Further growth inhibited by midazolam.
Freire-Garabal M et al 1993 Spain	Animal	Experimental	Alprazolam	influenza A virus	Alprazolam (1mg/kg/day) administered to stressed mice (mice exposed to chronic auditory stress).	Reduction in stress-induced increase of virus titres.

2.3 Benzodiazepines/Z-drugs and pneumonia risk

Pneumonia was the most common infection studied in relation to the assessment of the impact of benzodiazepine drugs on infection risk; the available evidence though is inconclusive. Table 2.2 summarises the existing data regarding the effects of benzodiazepines on pneumonia risk.

Benzodiazepines have recently been associated with a 54% increase in the occurrence of pneumonia compared to non-use of benzodiazepines (adj. OR 1.54; 95% CI 1.42, 1.67).[93] Sedative use has been associated with an over two-fold increase in the risk of pneumonia among residents of a long-term care facility.[94] Similarly, an increase in the risk of pneumonia was observed with the use of antipsychotic drugs.[95] However, it is difficult in such studies that use a composite exposure definition (e.g. use of sedatives or antipsychotics as a class of drugs without reports on individual drugs) to establish the individual effect of benzodiazepines on pneumonia risk.

On the other hand, a decrease in the incidence of pneumonia has been suggested among regular benzodiazepine users by Almirall et al.[96] However, this study determined drug exposure by questionnaires and thus is prone to reporting and recall bias. Furthermore, a similar study[97] as well as other different studies[98-100] did not establish statistically significant associations between benzodiazepines and pneumonia risk (Table 2.2.). A systematic review and meta-analysis

conducted in the initial phases of this doctoral research (presented in Appendix 2.0) found a 5% increased pneumonia risk with benzodiazepine use which lacked statistical significance (pooled OR=1.05, 95% CI 0.83, 1.32; I^2=87.0%).[101] A restricted analysis of only those studies assessed to be of high methodological quality according to the Newcastle Ottawa Scale[102](a validated tool for assessing the quality of observational studies) showed a 19% point estimate increase in pneumonia risk, but this also failed to reach statistical significance (OR 1.19; 95% CI 0.86, 1.66; I^2=91.5%). This underlines the need for additional studies to address the important question of whether benzodiazepines influence pneumonia risk.

Table 2.2: Studies investigating the association between benzodiazepines and pneumonia risk

Author, Year, Country	Study design and period	Study population	Definition of benzodiazepine use	Adjustment for confounders	Number (%) of pneumonia cases	Odds Ratio (95% CI)
Frank B et al (2014) UK	Nested case-control study 1998 to 2008	5938 patients in the Virtual International Stroke Trials Archive (VISTA)	Prescription benzodiazepines	National Institutes of Health Stroke Score, alteplase in patients with ischaemic stroke and age	779 (13.32)	0·99 (0·83–1·18)
†Iqbal U et al (2013) Taiwan	Nested case-control study -	Taiwan National Health Insurance Database	Prescription benzodiazepines in a number of specified periods e.g. 30, 60 and 90	None	56019/not specified	OR 0.46‡
Obiora et al (2012) UK	Nested case-control study 2001-2002	34661 patients from the general population in The Health Improvement Network (THIN)	Prescription benzodiazepines (e.g. current use was defined as the most recent prescription within 30 days before the pneumonia diagnosis date)	Age, sex, Ischemic heart disease, pulmonary disease, previous pneumonia episodes, Charlson's comorbidity index, alcohol consumption, depression, Psychosis, current smoking and socioeconomic status	4964 (14.32)	1.54(1.42-1.67)
Dublin et al (2011) ***f*** USA	Nested case-control study 2000-2003	3061 older adults (aged 65-94 years) in Group Health, an integrated health care delivery system	Prescription refill data (e.g. Current use was defined as having filled one or more Prescriptions between 5 and 60 days before pneumonia diagnosis date)	Age, sex, asthma, COPD, COPD hospitalisation, use of oxygen, corticosteroid use, use of opioids, furosemide, bronchodilators, CHF, CHF hospitalisation, stroke, number of outpatient visits in 1-year baseline period, need for assistance with ambulation or bathing and dementia	1039 (33.94)	**Current use** 1.08(0.84-1.47) **Amount of use** Heavy (≥150 TSD¶) 0.94 (0.60-1.47) Light (≤150 TSD¶) 1.27 (0.84-1.77)

Author, Year, Country	Study design and period	Study population	Definition of benzodiazepine use	Adjustment for confounders	Number (%) of pneumonia cases	Odds Ratio (95% CI)
Trifiro et al (2010) The Netherlands	Nested case-control study 1996-2006	1944 elderly patients (≥65 years) in the Dutch Integrated Primary Care Information, general practice database	Previous and concomitant use (within 3 months before date of first specific pneumonia symptom) of benzodiazepines with antipsychotic drugs	Year of birth, sex and index date (matching variables)	258 (13.27)	1.17(0.89-1.54)
Almirall et al (2008)/ 2013 (re-analysis) Spain	Population based ase-control study 1999-2000	2662 residents in the Eastern coast of Spain	Regular benzodiazepine treatment a year prior to the study	age, sex, primary centre, asthma, chronic bronchitis, upper airway infection in the last month, hospitalisation in the last 5 years, temperature change, educational level and flu vaccination	1336 (50.19)	Overall 0.94(0.64-1.10) Subgroup analysis 14-60 years: OR 0.53 (0.31- 0.91)
Knol et al (2008) The Netherlands	Nested case-control study 1985-2003	2706 elderly people in PHARMO record linkage system (an electronic pharmacy and hospital records database)	Prescription benzodiazepines within 6 months prior to pneumonia diagnosis date	None	543 (20.07)	1.30(1.10-1.60)‡
Vergis, E. N. et al (2001) USA	Case-control study 1997-1998	208 residents in a long- term care facility (HJ Heinz Division of the Veterans Affairs Medical Center)	Sedative use within 14 days prior to onset of pneumonia	admission date, level dependence, and duration of institutionalisation	104 (50.00)	2.60(1.20-5.40)
Almirall et al (1999) Spain	Population based case-control study 1993-1995	680 residents from a mixed residential-industrial urban area in Maresme county, Barcelona, Spain.	Regular benzodiazepine use in a year prior to the study	Age, sex, municipality, weight, smoking status, any other respiratory infection, previous confirmed pneumonia, diabetes, chronic bronchitis, diagnosed asthma, Lung tuberculosis, chronic liver disease, aminophylline use and Use of aerosols and plastic pear-spacers	205 (30.15)	0.46(0.23-0.94)

Note:

- CAP=community acquired pneumonia; COPD=chronic obstructive pulmonary disease; CHF=chronic heart failure; FEV1=forced expiratory volume in 1 second, EF=ejection fraction
- [‡]Crude Odds Ratio
- [†]Iqbal et al study did not specify the study period and the number of patients analysed
- [ƒ] Only Dublin et al considered benzodiazepine dose data
- [¶] TSD: Total standardized doses

2.4 Benzodiazepines and mortality risk

A number of studies have investigated benzodiazepines use and mortality. Table 2.3 summarises the relevant studies. Three recent population based studies show increased mortality with use of benzodiazepines in the general population.[103-105] However, in the elderly population in which hypnotics use is most prevalent, the increase in mortality risk does not reach statistical significance.[106-108] There are also concerns that other vulnerable groups of people, for instance those with chronic diseases, may particularly be more prone to the increased harm from benzodiazepines.

Frandsen et al. (2014) studied the association of benzodiazepines/benzodiazepine-like drugs and all-cause mortality in patients with Parkinson's disease (PD), and a matched group without PD. Their findings suggest similar effects of benzodiazepines/non-benzodiazepines on mortality risk in both groups of patients (benzodiazepines PD: HR 1.17; 95% CI 0.99-1.38, Non-PD: HR 1.39; 95% CI 1.29-1.51; benzodiazepine-like drugs PD: HR 1.33; 95% CI1.11 to 1.59, Non-PD HR 1.27; 95% CI 1.18 to 1.37).[109] However, a systematic review of literature by Amarasuriya et al.,[75] for mortality and benzodiazepines could not draw any definitive conclusions, citing methodological flaws in the existing literature. Similar flaws were encountered in this literature review and are discussed below.

Some of the definitions of benzodiazepine use lacked clarity, for instance the duration of benzodiazepine use was unspecified in three studies.[110-112] Such unclear and broad definitions increase the chances of misclassifying exposure status i.e. including non-users as users of benzodiazepines. In addition, over 5 studies reported the effect of benzodiazepines on mortality while combined with other drugs; commonly reported as benzodiazepine/antidepressants use, anxiolytics or hypnotics or sleeping medication/pills or sedatives (Table 2.3). This made it difficult to draw any inferences regarding benzodiazepines and mortality.

In some studies, self-reported medication use was analysed which is prone to recall and reporting bias.[112, 113] The extent of confounder control also varied with some studies undertaking complicated analyses including substantial number of variables (e.g. 31 covariates in a single model in Kripke et al 1998 study[114][Table 2.3]).

Despite the above limitations, the literature seems to suggest an increased risk of mortality with benzodiazepines use.[75]

Table 2.3: Summary of studies investigating benzodiazepine use and mortality

Author, Year, Country	Study design and period	Study population	Definition of BZP use	Confounder adjustment	Definition of mortality	Mortality estimate
Dupouy, J. et al (2014) France	prospective cohort study 2006 to 2012	114574 general population aged ≥18 years in the French reimbursement database EGB	New users of BZPs, with no BZPs nor antidepressants or non-BZPs anxiolytics/hypnotics in the past year	Year of birth, gender, clinical covariates	12 months all-cause mortality.	Adj. HR (95% CI) 1.26 (1.08-1.48) p=0.004
Weich S et al (2014) UK	prospective cohort study 1998 to 2013	104389 general population aged ≥ 16 years in the UK general practice database	Prescription benzodiazepines	Sex, age at study entry, sleep disorders, anxiety disorders, other psychiatric disorders, medical morbidity, and prescriptions for non-study drugs, alcohol, smoking and socioeconomic status.	All-cause mortality	Adj. HR(95% CI) 3.32 (3.19 to 3.45)
Ekström, MP. et al (2014) Sweden	Prospective cohort study 2005 to 2009	2249 COPD patients in the national Swedevox Register	Prescription benzodiazepines	age, sex, arterial blood gases, BMI, performance status, previous admissions, comorbidities, and concurrent drugs	All-cause mortality	Adj. HR(95% CI) 1.21 (1.05 to 1.39)
Frandsen R et al (2014) Denmark	Retrospective cohort study 1997 to 2007	5861 Parkinson's disease (PD) and 31,395 non-PD patients	Prescription benzodiazepines	age, gender, marital status	All-cause mortality	HR(95% CI) benzodiazepines PD: 1.17 (0.99 to 1.38) Non-PD:1.39 (1.29 to 1.51) BZP-like drugs PD: 1.33 (1.11 to 1.59) Non-PD 1.27 (1.18 to 1.37)
Jaussent I et al (2013) France	Prospective cohort study 1999 to 2011	6696 elderly people (≥65 years) in multi-site longitudinal French study.	Prescriptions and over-the-counter drugs (BZPs, zopiclone and zolpidem) used during the month preceding baseline, 2, 4, and 8-year follow-up period.	Study center, age, gender Socio-demographic and lifestyle characteristics, chronic disorders including cardiovascular pathologies, sleep and psychiatric disorders	All-cause mortality within 12- year follow-up	Adj. HR(95% CI) 1.10 (0.96;1.25)

Table 2.3 continued

Author, Year, Country	Study design and period	Study population	Definition of BZP use	Confounder adjustment	Definition of mortality	Mortality estimate
Obiora E et al (2012) UK	Prospective cohort study	29 697 controls and 4964 cases of community-acquired Pneumonia in a UK primary care patient database (THIN)	Prescription BZP (e.g. current use was defined as the most recent prescription within 30 days before the pneumonia index date)	Age, sex, Ischemic heart disease, pulmonary disease, previous pneumonia episodes, Charlson's comorbidity index, alcohol consumption, depression, Psychosis, current smoking and socioeconomic status	30-day and long-term all-cause mortality	HR(95% CI) 30-day mortality 1.22 (1.06- 1.39) long-term mortality 1.32 (1.19 - 1.47)
Kripke FD et al (2012) US	Prospective cohort study 2002 to 2007	10 529 patients and 23 676 controls from Geisinger electronic health records	Prescription hypnotics (BZP or Z-drugs)	age, sex, ethnicity, marital status, body mass index (BMI) and self-reported alcohol use and smoking status and comorbid diseases	All-cause mortality	Hypnotics HR(95% CI) Doses 0.4–18 /year 3.60 (2.92 to 4.44) Doses 18–132/ year 4.43 (3.67 to 5.36)
Gisev N et al (2011) Finland	Prospective cohort study 2000 to 2008	2224 older people (≥65 years) in the Finnish National Prescription Register	Prescription benzodiazepines	age, sex, antipsychotic drug use, and comorbidities	All-cause mortality	HR(95% CI) 1.01 (0.84 to 1.21)
¶Huybrechts KF et al (2011) British Columbia	prospective cohort study 1996 to 2006	10900 older patients ((≥65 years) in British Columbia nursing homes	Psychotropic prescription (e.g. atypical antipsychotics and benzodiazepines) during the first 90 days after admission to nursing home	age, sex, calendar year, level of care assigned at the time of admission to a nursing home and Charlson comorbidity index	Non-cancer deaths within 180 days after the index date	Adj .RR (95% CI) Benzodiazepines: RR 1.28 (1.04–1.58)
Van de Nadort et al (2009) The Netherlands	Retrospective cohort study January 1997 to February 2003	509 patients of LRTI aged ≥80 years from Utrecht GP research network database	Use of BZP or antidepressants for ≥1 week at the start of the LRTI episode	None	Hospitalisation or death within 30 days after LRTI diagnosis irrespective of the primary cause	Crude OR (95% CI) 1.2 (0.7 to 1.9)
Winkelmayer et al (2007) USA	Prospective cohort study 1997 to 2000	3630 patients in a national cohort of Dialysis Morbidity and Mortality study (DMMS wave 2)	Manual abstraction of medications use from the patient questionnaire	All covariates irrespective of significance in the model, which included; age, gender, race, education, marital status, smoking and co-morbidities	Mortality from any cause as reported by the US Renal Data System	HR (95% CI) 1.15 (1.00 to 1.31)

Table 2.3 continued

Author, Year, Country	Study design and period	Study population	Definition of BZP use	Confounder adjustment	Definition of mortality	Mortality estimate
Hausken et al (2007) Norway	Population-based Prospective cohort study Follow-up period=18 years	14951 patients aged 40-42 years from the general population	Self-reported use of anxiolytics or hypnotics Daily; every week, but not daily; less than every week; not used during last month	Painkiller use (daily/every week, <every week), number of cigarettes/day, marital status (married, not married), coffee (5þ, <5 cups/day), physical activity (seldom/never, intermediate/intensive), sick-leave/receiving rehabilitation benefit or disability pension (yes/no)	Deaths (from all causes) obtained from official statistics of the population of Norway	Daily use crude HR (95% CI); Men: 3.1 (2.0-4.8) Women: 2.7 (1.9 to 4.0) Adjusted HR(95% CI) Men: 1.5(0.9-2.7) Women: 1.7(1.1 to 2.6)
Trifiro G et al (2007) The Netherlands	Prospective cohort study 1996 to 2004	2385 dementia patients aged ≥ 65 years in the Integrated Primary Care Information (IPCI) database, a longitudinal general practice research database	Current use of BZP if the date of dementia diagnosis fell within the duration of treatment or within a maximum of 60 days after the end of the last prescription.	None	All-cause mortality	Crude OR (95% CI) 1.2 (1.0 to 1.5)
Fukuhara et al (2006) Japan	Prospective cohort study 1999 to 2004	1603 Japanese hemodialysis patients in DOPPS(an international observational study of hemodialyis patterns and associated outcomes in 12 countries)	Assessment of patients' medical record for BZP prescription given on or before entry into the study	Mental health index score, age, sex, time on dialysis, socio-economic factors, comorbid conditions, serum albumin, haemoglobin and dialysis dose	All-cause mortality	RR (95% CI) 1.27 (1.01-1.59)
Lwata et al (2006) Japan	Prospective cohort study I year follow-up	403 patients aged ≥ 85 years discharged from the acute care of a general hospital	BZP use	Comorbidites (Charlson Comorbidity index ≥2), six or more prescribed medication at discharge , NSAIDS drug use and history of delirium	All-cause mortality	HR (95 % CI): 1.64 (1.04 to 2.60)

Table 2.3 continued

Author, Year, Country	Study design and period	Study population	Definition of BZP use	Confounders adjustment	Definition of mortality	Mortality estimate
Hak E et al (2005) The Netherlands	Retrospective cohort study March 1998 to February 2003	455 Patients with pneumonia and non-pneumonia LRTI, aged ≥60 years from Utrecht GP research network database	Prior use of BZP/antidepressants	Age, sex, heart failure and Dementia/stroke (all identified as independent predictors of endpoint)	Hospitalisation or all-cause mortality within 30 days of LRTI diagnosis	Adjusted OR (95% CI) All patients (n=455): 1.89 (1.02 to 3.52) Pneumonia patients (n=229): 1.58 (0.78 to 3.22)
Vinkers et al (2003) Leiden, The Netherlands	Population-based Prospective cohort study Sept 1997 to Sept 2002	599 inhabitants of Leiden, aged ≥ 85 years	Annual assessment of computerised pharmacy registries with a 3 months' time frame. BZP use assumed if the duration of prescription was more than half of the 3 months period assessed	Sex, level of education, chronic disease, marital status, living arrangements, depression and cognitive impairment	All-cause mortality	Adjusted RR (95% CI) 0.68 (0.44 to 1.04)
Hogan et al (2003) Canada	Prospective cohort study 1990 to 1996	2914 senior citizens aged≥ 65 years from selected urban communities and institutions across Canada	BZP use	Affect, self-rated health, cognition, depression, function and self-rated health	All- cause mortality	Mortality in BZP users was 54.8% versus 53.2% in non-users at baseline (P value=0.48)
Kripke et al 1998 USA	Prospective cohort study 1982 to 1988	1,099,830 participants in the American Cancer Society's Cancer Prevention Study	Use of "Prescription sleeping pills" 30+ times in the past month	31 covariates including race,history of heart disease education, cancer and BMI	All-cause mortality	HR (95%bCI) Women Aged <50 years: 1.11 (0.76 to 1.63)

Table 2.3 continued

Author, Year, Country	Study design and period	Study population	Definition of BZP use	Confounders adjustment	Definition of mortality	Mortality estimate
Joseph KS et al (1996) Canada	Retrospective case-control study 1978 to 1987	131 cases of death or near death from asthma and 3930 matched non-cases aged between 5 and 54 years from a cohort of asthmatic patients with psychosis in the computerised health databases of the Canadian province of Saskatchewan	At least one prescription for a sedative in the 12 months preceding the index date	Area of residence, number of admissions to hospital in the previous year, numbers of canisters of inhaled fenoterol, inhaled salbutamol, inhaled corticosteroids, and other inhaled antiasthma drugs and numbers of prescriptions for nebulised salbutamol, oral salbutamol, oral corticosteroids, and theophylline	Death or near death from asthma	Relative risk(95% CI) Crude RR: 1.9(1.2 to 3.1) Adj. RR: 1.4(0.8 to 2.5)
Rumble and Morgan (1992) Australia	Prospective cohort study 5 year follow-up	1042 respondents aged ≥ 65 years from a longitudinal study of health, activity and lifestyle	Use of "sleep medication" categorised as Hypnotic drug use (use of medication with recognised hypnotic or sedative actions) or other drug use (including analgesics and other OTC medicines)	Gender, health risk, usual sleep duration	All-cause mortality	OR(95% CI) 1.20 (0.83 t0 1.73)

Note: ¶ Comparison patient group in the study by Huybretchts et al was atypical users of antipsychotic drugs

Chapter 3 : General methods

3.1. Introduction to this chapter

This research project utilised routinely collected patient care data. In this chapter, the database, study variables and an overview of study designs are presented. Detailed study-specific methods are described in subsequent chapters focusing on the individual studies that are part of this thesis.

3.2. The Clinical Practice Research Datalink

The main database used in this research was the Clinical Practice research datalink (CPRD). The CPRD, formerly known as the General Practice Research Database (GPRD), is a longitudinal database comprised of anonymised electronic medical records prospectively collected from over 600 general practices across the UK and covering approximately 6% of the UK population (http://www.cprd.com/). At the time of this study's data extraction in February 2012, 639 general practices were contributing data to CPRD for over 11 million anonymised patients.

General practitioners (GPs) in the UK health care system (The National Health Service [NHS]), are responsible for primary health care and specialist referrals, which means that referrals to and discharges from secondary care are also recorded in the general

practice electronic patient records systems. In addition, the primary care database is linked to other data sources for example hospital patient records (the Hospital Episode Statistics database [HES] and national death registry data (the Office of National Statistics [ONS]) which were utilised in this PhD research.

3.2.1. History of CPRD

The CPRD was set up in 1987 by a company known as Value Added Information Products (VAMP) Ltd at a time GPs started using computers to record patient data. At that time, free computers and system software were offered to general practices in return for generating anonymised patient data according to set standards.[115] This later changed to financial incentives to GPs for the data provided. CPRD has gone through a series of owners including Reuters Health Information which donated the database to the UK Department of Health. Currently, the database is overseen by the Medicines and Health Products Regulatory agency (MHRA) on behalf of the National Institute of Health Research (NIHR).[116]

3.2.2. Data collection and quality control

Data are collected from volunteering general practices which use the Vision clinical system software provided by In Practice Systems Ltd. The software that undertakes data collection is an integral part of the Vision practice software system. On joining CPRD, an initial data

collection of all available historical electronic medical records (EMR) on the practice computer is carried out. Subsequent data collection is done approximately monthly, on an incremental basis. CPRD has demonstrated a high level of completeness of clinical, diagnostic and prescription data according to validation studies.[117] It has set standards in terms of accuracy and completeness of data at a patient and practice level as a means of data quality control. Patients who fulfil the quality checks (e.g. presence of a valid registration status, age and gender) are labelled as 'acceptable' in the data while practices are assigned an 'up to standard' date (UTS date ["first date at which an individual practice's longitudinal data meets the CPRD-defined minimum standards for data recording quality"]).[118] The data collected from CPRD contributing practices are anonymised for confidentiality reasons. Each record in CPRD has an encrypted unique identification number for every individual and for the practice to which they belong. In addition, data fields with strong identifiers (e.g. name, title, address, NHS number and telephone number) are not collected. Table 2.1 presents the data collected for CPRD, extracted as text files that can be linked by a unique identification number. Medical history (including diagnoses, signs and symptoms) is recorded using the Read clinical coding system which can be cross-referenced to the International Classification of Diseases (ICD-9)[119], while the information on drug prescription is coded according to chapters of the British National Formulary (BNF).[41]

Table 3.1: CPRD data structure

Data category (file)	Description
Clinical	Medical history details including diagnoses and symptoms
Additional Clinical details	Patient-lifestyle factors e.g. smoking and alcohol status
Therapy	Prescriptions for drugs and appliances
Patient	Basic patient demographics and registration details
Practice	Practice details including data collection date
Staff	Staff identification number, gender and role e.g. GP registrar, practice nurse
Consultation	Details of a GP consultation including type of a consultation e.g. Emergency, normal Surgery Consultation
Referral	Patient referrals normally for secondary care
Immunisation	Records of immunisations including components of an immunisation and the route of administration
Test	Records of test data including type of test used e.g. laboratory tests, pathology

3.3. CPRD-linked data

Although CPRD (general practice data) covers the majority of patient care data, some may still be missed for example in instances when patients walk in to hospital or accident and emergency departments without a referral from their GPs. The CPRD linked data therefore offer a means to address such shortfalls in data coverage. CPRD patient level data are linked to external data sources by a trusted third party using patient identifiers, e.g. NHS number, date of birth and gender. Currently linked data are only available for practices in England although CPRD covers all the four countries in the UK (England, Scotland, Wales and Northern Ireland). England however has the largest proportion of practices in the UK (for example as of October 2010, England had 8,230 general practices, Scotland 1,025, Wales 499 and Northern Ireland 358).[120] In addition, general practices consent to take part in the CPRD linkage schemes; therefore CPRD linked data represent a subset of all the data in CPRD available from English practices. CPRD linked Hospital Episode Statistics and Office of National Statistics mortality data were utilised in this PhD research.

3.3.1 Hospital Episodes Statistics data

The Hospital Episode Statistics (HES) database records all admissions to or attendances at NHS hospitals in England since 1989. HES also contains data on private patients and those living outside England but

treated in NHS hospitals as well as for patients cared in treatment centres funded by NHS.[121]

Although HES contains data on admitted patient care, accident and emergency attendances and outpatient appointments, at the time of data acquisition for this PhD research, only inpatient data were linked to CPRD. These data are arranged in files relating to hospitalisations and episodes. A hospitalisation is defined as the total period of inpatient hospital stay from admission to discharge whereas an episode is a time period within a hospitalisation corresponding to the period of a patient's care under a single consultant. Thus a single hospitalisation may have more than one episode.

HES linked data provided by CPRD mainly contains patient details (e.g. age, sex, date of admission and discharge) and information on every hospitalisation and episode for the patient including the medical diagnoses and procedures undertaken per episode and during the entire hospitalisation. Diagnoses are coded using ICD codes and up to 20 diagnoses may be recorded per episode.[122]

3.3.2 Office of National Statistics (ONS) mortality data

It is a legal requirement to register all deaths occurring in England and Wales with the local registrar's office. Death registrations provide the most complete data source for deaths. Information on the registered deaths is passed onto ONS on a weekly basis.[123] The details collected when death occurs include date of death, place of death,

and underlying cause of death (disease or injury which initiated the chain of events directly leading to death) and up to 15 other possible causes of death. Cause of death data are coded using the ICD coding system.[124] ONS-CPRD linked data were obtained from CPRD as a separate tab delimited text file which was linked to the relevant CPRD patient cohort file using the encrypted patient identifier.

3.4. Ethics and Data extraction for the PhD research

This study was approved by the independent Scientific Advisory Committee for Medicines & Healthcare products Regulatory Agency (MHRA) database research (Reference number: 11_098RA). It utilised the on-line CPRD data available for download by CPRD licence holders.

Data were extracted from the February 2012 version of the CPRD database using the available on-line data extraction tools. These include; disease and drug coding dictionaries (Medical and Product browsers respectively), the 'Define' tool (used to query the database in order to 'define' a patient cohort of interest) and 'Extract' tool (that enables extraction of data for a specified list of patients). Asthma and influenza like illness (ILI) codes (Appendix 3.1 and 3.2 respectively) were obtained from the CPRD medical browser (this allows searching for Read codes or diagnoses using search terms). Using the 'Define' tool, patients with asthma or ILI Read codes during the study period (January 2005 to December 2011) were identified. Using the 'Extract'

tool, their data were obtained in separate text files as per structure illustrated in Table 3.1. Read codes for asthma and ILI which were included in the study analyses are presented below in Table 3.2 and Table 3.3.

Codes for the main exposures (benzodiazepines and zopiclone [Appendix 4.0]) were searched for using the CPRD product browser (this allows searching for BNF or therapeutics codes using search terms). The Medical and Product browsers were also searched for disease and drug covariates codes respectively. Deprivation data (area-level deprivation measure based on patient's residence postcode as measured using census data) were requested from CPRD by providing them with a list of study patients.

Table 3.2: Frequency of asthma Read codes in the asthma study analysis sample

Read term	Read code	Percent
Asthma	H33..00	95.34
Asthma NOS	H33zz00	1.95
Bronchial asthma	H33..11	0.8
Asthma unspecified	H33z.00	0.59
Extrinsic (atopic) asthma	H330.00	0.4
Childhood asthma	H330.12	0.31
Late-onset asthma	H33z200	0.21
Intrinsic asthma	663V300	0.09
Severe asthma	H331.00	0.09
Late onset asthma	H331.11	0.08
Mixed asthma	H332.00	0.04
Brittle asthma	H334.00	0.04
Extrinsic asthma NOS	H330z00	0.03
Extrinsic asthma without status asthmaticus	H330000	0.01
Intrinsic asthma NOS	H331z00	0.01
Intrinsic asthma without status asthmaticus	H331000	0

Table 3.3: Frequency of ILI Read codes in the ILI study analysis sample

Read term	Read code	Percent
Upper respiratory tract infection NOS	H05z.11	76.1
Acute upper respiratory tract infection	H051.00	11.99
Viral upper respiratory tract infection	H05z.12	5.81
Influenza	H27..00	4.01
Influenza-like symptoms	16L..00	0.97
Influenza NOS	H27z.00	0.56
Influenza like illness	H27z.12	0.28
Influenza A (H1N1) swine flu	H2A..11	0.24
Influenza with respiratory manifestation	H271z00	0.02
Influenza due to Influenza A virus subtype	H2A..00	0.01

3.5 Data processing

The data were downloaded as tab delimited text files and subsequently imported to Stata version 12 for data cleaning, manipulation and for all the analyses. Additional data checks to supplement those undertaken by CPRD were performed to check for data anomalies and where found, the respective patients were excluded. These checks included: patient disease diagnosis date anomalies e.g. diagnosis date recorded before patient registration date, anomalies in patient registration dates e.g. start of registration occurring after end of registration (Appendix 5.0: Selection strategy for study subjects)

3.6. Key study variables

3.6.1. Primary outcome variables

There were five main outcomes of interest; asthma exacerbation and subsequent mortality for the asthma study presented in chapter 4,

all-cause mortality in the non-asthma study presented in chapter 5 and ILI-related pneumonia and ILI-related mortality for the ILI study presented in chapter 6. The definitions of these outcome variables are briefly discussed below.

Exacerbation of asthma

Using Read codes specific to asthma exacerbation (Appendix 3.1), all recorded exacerbations of asthma were identified from the asthma patient cohort. In patients with more than one asthma exacerbation, the first exacerbation was considered the outcome of interest.

ILI-related pneumonia

From the cohort of patients with a recorded GP diagnosis of ILI between January 2005 and December 2011, diagnoses of pneumonia were identified using relevant Read codes (Appendix 3.3). Pneumonia codes included in the analysis are presented below in Table 3.4. ILI-related pneumonia was defined as pneumonia within 30-days of an ILI diagnosis.

Mortality

Mortality in the asthma study was defined as all-cause mortality subsequent to asthma exacerbation whereas deaths within 30-days of ILI diagnosis denoted ILI-related mortality in the ILI study. In the non-asthma study, the outcome of interest was all-cause mortality during the study period.

Table 3.4: Frequency of Read codes for pneumonia included in the ILI-study analysis sample

Read term	Read code	Percent
Other specified pneumonia or influenza	H2y..00	30.62
Pneumonia due to unspecified organism	H26..00	26.41
Pneumonia or influenza NOS	H2z..00	9.67
Bronchopneumonia due to unspecified organism	H25..00	8.59
Lobar (pneumococcal) pneumonia	H21..00	5.46
Lobar pneumonia due to unspecified organism	H260.00	4.48
Pneumonia and influenza	H2...00	3.4
Basal pneumonia due to unspecified organism	H261.00	2.51
Atypical pneumonia	H28..00	1.97
Bacterial pneumonia NOS	H22z.00	1.61
Chest infection - unspecified bronchopneumonia	H25..11	1.16
Pneumonia due to other specified organism	H23..00	0.54
Other bacterial pneumonia	H22..00	0.45
Interstitial pneumonia	H20..00	0.27
Pneumonia – Legionella	H223.00	0.27
Pneumonia due to streptococcus	H22y200	0.27
Viral pneumonia	H56y100	0.27
Chest infection - other bacterial pneumonia	H20z.00	0.18
Influenza with pneumonia	H22..11	0.18
Pneumonia due to mycoplasma pneumonia	H23z.00	0.18
Pneumonia due to specified organism NOS	H270.00	0.18
Viral pneumonia NOS	SP13100	0.18
Abscess of lung with pneumonia	A3BXA00	0.09
Chest infection - influenza with pneumonia	H20..11	0.09
Chest infection - pneumococcal pneumonia	H202.00	0.09
Chest infection - viral pneumonia	H21..11	0.09
Herpes simplex pneumonia	H220.00	0.09
Hypostatic pneumonia	H246.00	0.09
Mycoplasma pneumoniae [PPLO] cause/disease	H270.11	0.09
Pneumonia due to klebsiella pneumonia	H470312	0.09
Pneumonia due to parainfluenza virus	H530300	0.09
Pneumonia with aspergillosis	H540000	0.09

3.6.2. Exposure variables

Benzodiazepines and zopiclone prescriptions were the study drugs of interest. A search for these drugs was carried out within the patients' prescription records using the relevant therapeutic codes (Appendix 4.0: Prodcodes for benzodiazepines and zopiclone) and a prescription

for these drugs was considered a proxy marker for their use. The benzodiazepines available as prescription drugs in the BNF were considered both as a class and individual drugs. Table 3.5 presents a summary of the benzodiazepines included in the analysis of the respective studies.

Table 3.5: Main drug exposure of interest

Drug family	Generic drug name	Drug distribution in the study (%)	
		Asthma study	**ILI study**
Benzodiazepines	**Diazepam**	**65.66**	**66.27**
	Temazepam	**23.87**	**25.66**
	Lorazepam	**2.79**	**2.44**
	Chlordiazepoxide hydrochloride	2.58	2.30
	Nitrazepam	2.48	2.09
	Clonazepam	1.79	1.02
	Midazolam hydrochloride	0.77	0.17
	Alprazolam	0.07	0.06

Note: Highlighted in bold are the most frequent individual benzodiazepines earmarked for subgroup analyses

3.6.3. Covariates

3.6.3.1. Co-morbidity

The Charlson's comorbidity index score was adapted as a measure of disease burden. This is a validated weighted comorbidity index, based on a number of medical diagnoses identified by Charlson et al. as predictors of 1-year mortality.[125] Specifically, this considers congestive heart failure, myocardial infarction, peripheral vascular disease, dementia, cerebrovascular disease, chronic pulmonary disease, connective tissue disease, ulcer disease, liver disease, renal disease, diabetes, hemiplegia, cancers and AIDS. Among the chronic pulmonary diseases stipulated in Charlson's comorbidity index, codes

relating to asthma were excluded in the asthma study. Each of the co-morbidities in this index is assigned a weighted score, with the total score as an indicator of the patient's disease burden. Other comorbid conditions controlled for include anxiety, stress, insomnia, depression and psychosis which were identified as important confounders in previous related studies.[104, 126] Using relevant Read codes, data on comorbidities of interest were ascertained from patient records. Only those records before the index date (asthma exacerbation diagnosis date for cases and the corresponding control in the asthma study and ILI diagnosis date in the ILI study) were considered. Chronic comorbidities were allowed to have occurred at any time whereas acute conditions were considered present if recorded within a year prior to the index date. In the non-asthma mortality study, records of acute conditions considered were only those within a year prior to study entry date or occurring during follow-up whereas chronic conditions recorded at any time before study entry date or during follow-up were considered.

3.6.3.2. Other drugs

Other key drugs considered include: statins, corticosteroids (oral, inhaled, nasal and nebulised), opioids and beta-2 agonists. Exposure to these drugs was defined as having a prescription of the respective drug within the 30 days prior to the date of asthma exacerbation for

the case-control study of benzodiazepine and asthma exacerbation. Exposure to covariate drugs (and the primary exposure i.e. BZPs) was defined as current when prescriptions were issued in the 30 days prior to the outcome of interest.

3.6.3.3. Smoking

Data recorded on smoking before cohort entry in the non-asthma study and before the index date in the asthma study and ILI study were ascertained from patient records. In patients with more than one entry of smoking status, the latest record prior to cohort entry in the non-asthma study and before the index date in the asthma and ILI study was retained in the analyses. Subjects were categorised as current smokers and non-smokers. Ex-smokers were included in the non-smokers category.

3.6.3.4. Alcohol consumption

Alcohol consumption in units of alcohol consumed per week was determined from patient records. Only those entries occurring before the index date (i.e. asthma exacerbation diagnosis date for cases and the corresponding control in the asthma study, ILI-diagnosis date in the ILI study) or study entry date in the non-asthma study were considered. In patients with more than one record, the latest record prior to study entry in the non-asthma study and before the index date in ILI and asthma study was retained in the analyses. A categorical variable to signify alcohol consumption levels was created

(at/above the recommended weekly consumption versus below the recommended threshold). UK weekly recommended alcohol consumption limits were used to define thresholds: <14units/week for women and <21units/week for men.[127]

3.6.3.5. Body mass index

Body mass index (BMI), as recorded by general practitioners was also considered a potential confounder. Data entries on BMI before index date in the asthma study and ILI study and cohort entry date in the non-asthma study were ascertained from the patient records. In patients with more than one BMI record, the latest record prior to study entry in the non-asthma study and before the index date in ILI and asthma study was retained in the analyses. BMI was categorised using WHO cut-off points: BMI less than 18.5-underweight, BMI from 18.5 to 24.9-normal, 25 to 29.9-overweight and BMI >30 as obesity.[128]

3.6.3.6. Socioeconomic status: Index of Multiple Deprivation

Socioeconomic status appears to be linked to morbidity and mortality[129], thus socioeconomic disadvantage was controlled for using the Index of Multiple Deprivation (IMD) score quintiles. The IMD is a measure of area-level deprivation that combines weighted scores in seven deprivation domains namely, income, employment, health, education, housing and services, living environment and crime. These are available at the 'lower layer super output area' level

(small geographical areas with a population of approximately 1,500 people). The IMD was grouped into quintiles of deprivation with the first quintile corresponding to the least deprived and the fifth quintile to the most deprived. IMD data are only available for a subset of English practices consenting to CPRD data linkages and relate to patient residence postcodes. Therefore not all patients included in the analysis had IMD data and a category for missing IMD data was created.

3.7. Overview of study designs

An overview of the study designs used in this research project is presented below. Detailed methods relating to patient selection and study conduct will be discussed in subsequent chapters dedicated to each study objective.

3.7.1 Asthma exacerbation case-control study

A nested case-control study design was used to investigate the association between benzodiazepines and the occurrence of asthma exacerbations. The data for the main analyses were obtained from CPRD. CPRD-HES linked data was used in the sensitivity analyses to quantify exacerbations missed from CPRD primary care records and account for the potential impact of these missed diagnoses on the study findings.

The study population for this case-control study included all patients diagnosed with asthma during the period 2005 to 2011. Cases were asthma patients in the CPRD database with a diagnosis of asthma exacerbation within the study period (2005-2011). Identification of cases was done by using specific medical Read codes corresponding to a diagnosis of exacerbation of asthma (Appendix 3.1). Only the first recorded diagnosis of exacerbation within the study period was considered for each case. Asthma patients, who had not experienced an exacerbation during the study period, were identified as potential controls. These were individually matched to cases by age at index date (±3 years), sex and general practice (matching on general practice was performed to account for differences in prescribing policies and patient management). A maximum of six controls were matched to each case to give sufficient sample size for stratified analyses.

3.7.2. Mortality subsequent to asthma exacerbation study

A cohort study design was used to investigate the association between benzodiazepines and all-cause mortality subsequent to asthma exacerbations. CPRD and ONS data were utilised in the main analyses while CPRD-HES linked data were employed to identify hospitalised exacerbations (as a proxy measure for severity of asthma exacerbations) for the sensitivity analyses. The study population for this cohort study comprised of asthma exacerbation cases from the

case-control study above, identified from CPRD. For patients with death records in both CPRD and ONS mortality data, the date of death was preferentially determined from ONS data if in conflict. Death data for the remaining subjects were obtained solely from CPRD records (as ONS mortality data are available only for subjects registered with English practices that consent to CPRD-ONS data linkage). Patients were followed from the diagnosis date of asthma exacerbation until death or when they were censored (i.e. the end of the study period, date of the last CPRD data collection from the general practice and date of patient's transfer out of the practice).

3.7.3. Mortality in patients without asthma study

A prospective cohort study design was employed to determine the association between benzodiazepine use and all-cause mortality during the study period. Data utilised in this study were from CPRD and ONS. The study population comprised of patients without a diagnosis of asthma in CPRD. Like in the mortality asthma study above, death data was obtained both from CPRD and ONS. The follow-up started at the point of study entry (defined as the latest of following dates: study start date [01/01/2005], patient's registration date with their current practice and the date the practice first satisfied CPRD data recording quality checks) until death or censorship (end of study period [31st December 2011] or the general practice's last data collection date or transfer out of practice date' which ever happened earliest).

3.7.4 ILI-related pneumonia study

This study used primary care data from CPRD for all its analyses. The study population comprised patients with a clinical diagnosis of ILI (Table 3.3). The cohort study followed-up patients with ILI and investigated the association between benzodiazepine use and ILI-related pneumonia. ILI-related pneumonia was defined as pneumonia within 30-days following an ILI diagnosis.

3.7.5 ILI-related mortality study

As in the case of the ILI-related pneumonia, this was a cohort study which followed-up ILI patients and investigated the association between benzodiazepine use and ILI-related mortality. Primary care data from CPRD were used for the main analysis and mortality data from ONS were used for validation of death records. ILI-related mortality was defined as all-cause mortality within 30 days following an ILI diagnosis.

3.8. Statistical analyses

Conditional logistic regression was used to assess the association between benzodiazepines and exacerbation of asthma with results presented as unadjusted (OR) and adjusted matched odds ratios (adj mOR) and 95% confidence intervals (CI). The association between benzodiazepines and the outcomes (ILI-related pneumonia and mortality) were investigated using Cox regression. Proportional

hazards assumptions were checked for each of the drug exposures using log-log plots and the global test for proportional hazards. Kaplan-Meier survival curves were plotted for all-cause mortality over the entire study period for each of the drug exposures by status of use. Results of the survival analysis are presented as unadjusted (HR) and adjusted hazard ratios (adj HR) and 95% CI.

The following model building strategy was used to construct two multivariable models for each association investigated: Model 1 included all covariates that were significantly associated with both the outcome and the exposure variable ($p<0.05$) and Model 2 included all *a priori* confounders. Collinearity between variables included in the multivariable models was assessed by examining the variance inflation factor (VIF) values. A VIF value over 10 or a Tolerance (1/VIF) value lower than 0.1 indicated collinearity. Possible modification of the effect of benzodiazepines on various outcome measures by comorbidity burden, depression, anxiety, stress and psychosis (interaction terms) were assessed using the likelihood ratio test (LRT). The significance level in LRT was determined at $P<0.05$.

Chapter 4 : Association between benzodiazepine use and exacerbations and mortality in patients with asthma: A matched case-control and survival analysis

4.1 Introduction to this study

As established earlier in the literature review chapter (Chapter 2), epidemiological evidence for the effect of benzodiazepines/zopiclone on asthma outcomes is almost non-existent. Only one study was retrieved which assessed the impact of sedatives on mortality in asthma patients (Table 2.3), making it difficult to ascertain the individual effect of benzodiazepines/zopiclone.

This chapter presents the first epidemiological study assessing the association between benzodiazepines and asthma exacerbations and subsequent mortality in patients with asthma. To place this study in perspective, the background includes the evidence from laboratory studies on the role of GABAA receptors (the site of action for benzodiazepines/zopiclone) in the pathogenesis of asthma. The findings of this study have been published in a peer-reviewed journal (Appendix 6.0).

4.2 Background

Benzodiazepines may be used in the treatment of severe anxiety that presents during asthma attacks.[130, 131] However they are not innocuous; accumulating evidence suggests they may cause harm,[75,

[132] including increased mortality in the general population[104] and risk of cancer.[105] Benzodiazepines may also increase the risk of infection[58, 74, 133]

The safety of these drugs requires specific evaluation in the context of asthma given the putative role of GABAA receptors, the target site for benzodiazepine/zopiclone action, in the pathogenesis of asthma. In vitro, GABAA receptor activation appears to oppose bronchoconstriction by enhancing bronchial smooth muscle relaxation which may assist in the remediation of asthma exacerbations.[134] However, such an effect has been shown to be restricted to the alpha sub-units of GABAA receptors that are not sensitive to benzodiazepines.[134] On the other hand, there is increased expression of benzodiazepine-sensitive GABAA receptors on alveolar macrophages as demonstrated in allergen-induced asthma in mice.[58] Augmented GABAergic signalling, such as through benzodiazepine treatment, impairs immune responses through intracellular acidosis of monocytes and macrophages, leading to increased mortality in animal models of infection,[58, 83] supported by observations in humans.[104, 126] Furthermore, the airway epithelial cells in asthma show increased expression of GABAA receptors and potentiates mucus overproduction, a prominent pathological change in asthma exacerbation.[135] Given the intrinsic role of the pulmonary GABAergic system in the pathogenesis of asthma and accumulating data on potential harms of benzodiazepines especially from infections, it

seems prudent to evaluate the safety of GABAergic drugs in asthmatic patients. Thus, the hypothesis being tested in this study is that benzodiazepines and zopiclone influence the occurrence of asthma exacerbations, and mortality following asthma exacerbation.

4.3 Methods

4.3.1 Data source

Data were extracted from the Clinical Practice Research Datalink (CPRD) in February 2012. In addition, linkage data were obtained from HES to validate hospital attendance due to exacerbations, ONS (for validation of death data) and deprivation data were also separately requested.

4.3.2 Sampling frame

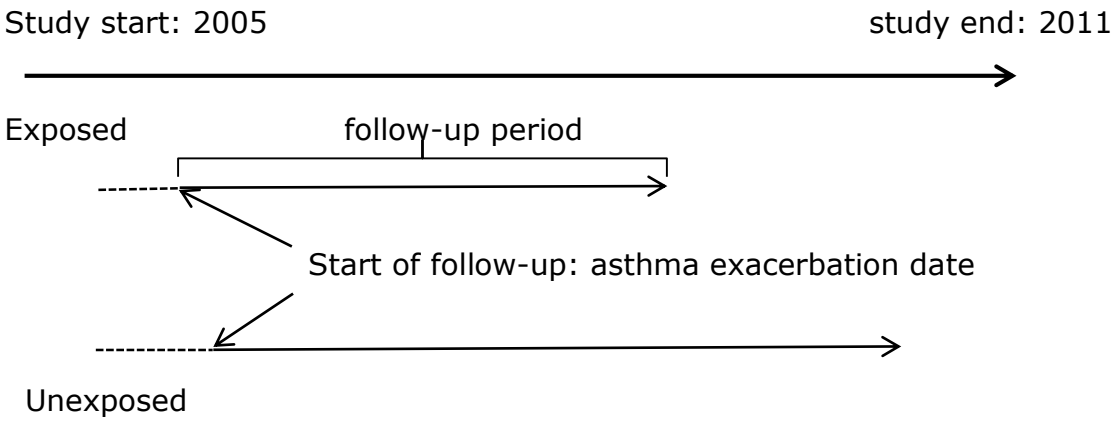
People with a diagnosis of asthma (n=225,543) were identified using relevant diagnostic codes (Appendix 3.1: Asthma Read codes) which map on to the international classification of disease (ICD-9) codes, for the study period 01/01/2005 through 31/12/2011. The selection process is summarised in Appendix 5.1.

4.3.3 Outcome definition

The primary outcome for the nested case-control analysis was physician diagnosed asthma exacerbation (cases), identified using relevant Read codes (Appendix 3.1: Asthma Read codes). Hospital data (CPRD-HES linked) were used to identify missing exacerbation

records (if for instance, the patient reported directly to hospital emergency services following an exacerbation) using the ICD 10 code for status asthmaticus (J46). HES data were also searched for a record of hospitalisation occurring within 14 days of a primary care diagnosis of asthma exacerbation as a proxy for severity of exacerbation. The date of the first recorded diagnosis of asthma exacerbation during the study period was designated the *index date*. All asthma patients, in the study population, who had not experienced an exacerbation during the study period (2005-2011), were identified as potential controls. These were individually matched to cases by age at index date (±3 years), sex and general practice (matching on general practice was performed to account for differences in prescribing policies and patient management). A maximum of six controls were matched to each case to yield a sufficient sample size for stratified analyses. The sample size was calculated using the Dupont Power and Sample size program (Appendix 7.0: Sample size calculations).[136] The secondary outcome of interest was all-cause mortality following a diagnosis of asthma exacerbation. Death (all-cause) occurring within 30-days of an asthma exacerbation was considered as being related to the asthma exacerbation in keeping with the convention used in previous pneumonia mortality studies.[126, 137, 138] Mortality was investigated using survival analysis and included only those patients diagnosed with an asthma exacerbation (Appendix 5.1: Patient selection flow chart).

Diagrammatic representation of the survival analysis cohort for mortality subsequent to asthma exacerbation



Note: BZP exposure was ascertained before asthma exacerbation (as illustrated below in section 4.3.4).

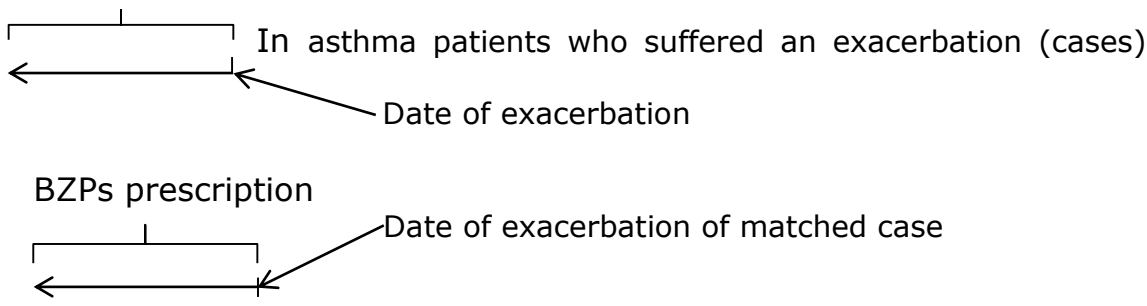
Observations were censored if patient transferred out of practice (lost to follow-up) or follow-up was completed before event (death) occurred.

4.3.4 Exposure definition

Data were extracted for all recorded prescriptions of benzodiazepines and zopiclone. The most recent prescription prior to the asthma exacerbation diagnosis date (or the index date of the matched case for controls) was retained. Benzodiazepines were considered both as a class and as individual drugs. The three most frequently used individual benzodiazepines in the study population, namely, diazepam, temazepam and lorazepam (General methods Chapter 3: Table 3.3) were also identified for *a priori* stratified analyses, representing long-acting, intermediate and short-acting agents respectively. Exposure was considered current when the most recent prescription was within the 30 days prior to the index date. The 30-day prescription window was chosen as the most commonly

prescribed oral benzodiazepines in the UK typically have a 20-28 tablet pack-size.[41] Further, it was reasoned that long-term regular users of benzodiazepines would have repeat monthly prescriptions from their general practitioner and therefore, the presence of a repeat prescription was used as a proxy indicator of long term drug use. Prescriptions in the period 31 to 90 days and >90 days before index date were classified as recent and past exposures respectively. No evidence of prescribing at any point in the patient's past primary care records defined 'non users'.

Diagrammatic representation of exposure ascertainment for the case-control analysis



4.3.5 Potential confounders

A validated weighted, comorbidity index (Charlson's comorbidity index) was adopted as a measure of comorbidity burden.[125] Specifically, this considers congestive heart failure, myocardial infarction, peripheral vascular disease, dementia, cerebrovascular disease, chronic pulmonary disease, connective tissue disease, ulcer disease, liver disease, renal disease, diabetes, hemiplegia, cancers and AIDS. In addition, we adjusted for depression, psychosis, stress and anxiety, to account for any confounding by indication (i.e. factors that could trigger a prescription of benzodiazepines/zopiclone).[104, 126] Other potential confounders considered included age, sex, current smoking (most recent record), alcohol consumption (measured as units of alcohol consumed per week and categorised according to the UK weekly recommended alcohol consumption limits; <14units/week for women and <21units/week for men), socioeconomic status (measured using Index of Multiple deprivation (IMD) score quintiles)[139] and body mass index (as recorded by general practitioners and categorised according to WHO guidelines).[128] Current exposures (as defined previously) to statins, opioids, beta-2 agonists and topical respiratory corticosteroids (oral, inhaled, nasal and nebulised) were also considered.

4.3.6 Statistical analyses

Conditional logistic regression was used to investigate the association between drug exposure and asthma exacerbation. Cox regression modelling was used to assess the association between drug exposure and mortality following asthma exacerbation. Validity of the proportional hazards (PH) assumption was tested using log-log plots and the global test for proportional hazards.

Two multivariable models were constructed to test the hypothesis for each exposure variable: Model 1 included all covariates that were significantly associated with both asthma exacerbation and benzodiazepines ($p<0.05$) and Model 2 included all potential confounders as listed in the previous section. A third model was planned to include all covariates that were significantly associated with both exposure and outcome ($p<0.05$) and modified the unadjusted effect of the exposure of interest on the outcome by more than 10% in either direction. This model was not implemented as none of the covariates satisfied the selection criteria. Missing data were included in the analysis as dummy variables. Interaction with Charlson's Comorbidity Index, depression, psychosis, anxiety and stress was tested using the likelihood ratio test and where a statistically significant interaction was found the multivariable analysis was repeated, stratified by the interaction variable categories.

To quantify the proportion by which the incidence of asthma exacerbation among asthma patients would reduce if exposure to

benzodiazepines was eliminated,[140] the adjusted attributable fraction (AF) among the exposed and the adjusted population attributable fraction (PAF) were determined using the user-generated package 'punaf' in Stata.[141, 142] We also calculated the unadjusted number needed to harm (the inverse of the absolute risk increase).[140] All statistical analyses were conducted in Stata version 12.

4.4 Results

4.4.1 Characteristics of study participants

A total of 131,642 patients were included in the main analysis after excluding patients with anomalies in key dates e.g. registration and death dates (Appendix 5.1: Patient selection flow chart). Of these, 75,616(57.44%) were females, 40,258(30.58%) aged below 18 years and 25,895(19.67%) had an asthma exacerbation of which nearly 8% (2,063) were severe. Benzodiazepine exposure was noted in 20,725 patients, comprising 15.74% of the study population overall. Patients with asthma exacerbation were more likely to be from more deprived settings, have a diagnosis of depression, be current smokers, overweight or obese and to have had a prescription of corticosteroids, opioids and beta-2 agonists (Table 4.1).

Table 4.1: Characteristics of asthma patients with and without asthma exacerbation (n=131,642)

Characteristic	Exacerbation of asthma		Crude OR (95% CI)	P Value
	No (n=105,747)%	Yes (n=25,895) %		
Age (years)				

Characteristic	Exacerbation of asthma		Crude OR (95% CI)	P Value
	No (n=105,747)%	Yes (n=25,895) %		
<18	32311 (30.56)	7945 (30.68)	†	
18-44	37089(35.07)	8640(33.37)		
45-65	24595(23.26)	6200 (23.94)		
>65	11752 (11.11)	3110(12.01)		
Gender				
Male	45205 (42.75)	10821(41.79)	†	
Female	60542(57.25)	15074(58.21)		
IMD score quintiles				
1 (least deprived)	13898(13.14)	2990 (11.55)	1	
2	14001 (13.24)	3478 (13.43)	1.18(1.11-1.26)	P trend < 0.001
3	12390 (11.72)	3018 (11.65)	1.18(1.10-1.26)	
4	12750(12.06)	3219(12.43)	1.25(1.16-1.33)	
5 (most deprived)	11884**(**11.24**)**	3014(11.64)	**1.29(1.20-1.39)**	
Missing	40824(38.61)	10176(39.30)	-	
Current smoking				
No	63522 (60.07)	17260 (66.65)	1	
Yes	16720**(**15.81)	4753 **(**18.35)	**1.12(1.08-1.17)**	< 0.001
Missing	25505(24.12)	3882(14.99)	-	
Alcohol consumption				
≤ alcohol weekly limit	31262 (29.56)	8011 (30.94)	1	
> alcohol weekly limit	3953 (3.74)	943(3.64)	0.93(0.86-1.00)	0.059
Missing	70532(66.70)	16941 (65.42)	-	
Anxiety				
No	104433(98.76)	25446(98.27)	1	
Yes	1314(1.24)	449(1.73)	**1.38(1.24-1.55)**	<0.001
Stress				
No	105275(99.55)	25734(99.38)	1	
Yes	472(0.45)	161(0.62)	**1.40(1.16-1.68)**	<0.001
Depression				
No	92720 (87.68)	21786(84.13)	1	
Yes	13027 (12.32)	4109 **(**15.87)	**1.34(1.29-1.40)**	< 0.001
Psychosis				
No	105418 (99.69)	25788(99.59)	1	
Yes	329 (0.31)	107(0.41)	**1.28(1.02-1.61)**	0.030
Sleeping problems				
No	99123(93.74)	23671(91.41)	**1**	
Yes	6624(6.26)	2224(8.59)	**1.38(1.31-1.46)**	<0.001
Charlson's Comorbidity index score				
0	91472(86.50)	21776(84.09)	1	
1-2	11189(10.58)	3286(12.69)	**1.20(1.15-1.26)**	P trend < 0.001
3-5	2710(2.56)	773(2.99)	**1.13(1.03-1.24)**	
>5	376(0.36)	60(0.23)	**0.60(0.45-0.79)**	
BMI				
Normal weight	24013(22.71)	5733(22.14)	1	
Underweight	1679(1.59)	410(1.58)	0.94(0.84-1.06)	P trend < 0.001
Overweight	19429(18.37)	5292(20.44)	**1.17(1.12-1.22)**	
Obesity	16195 (15.31)	5306(20.49)	**1.41(1.35-1.47)**	
Missing	44431(42.02)	9154(35.35)	-	

Characteristic	Exacerbation of asthma		Crude OR (95% CI)	P Value
	No (n=105,747)%	**Yes (n=25,895) %**		
Corticosteroids‡ (nasal, oral, inhaled and nebulised)				
No	104024(98.37)	23430(90.48)	1	
Yes	1723(1.63)	2465(9.52)	**6.32(5.91-6.75)**	< 0.001
Opioids‡				
No	105394(99.67)	25746(99.42)	1	
Yes	353(0.33)	149(0.58)	**1.65(1.35-2.02)**	< 0.001
Beta-2-agonists‡				
No	103468(97.8)	23042(88.98)	1	
Yes	2279(2.16)	2853(11.02)	**5.61(5.28-5.95)**	< 0.001
Statins‡				
No	105321(99.60)	25779(99.55)	1	
Yes	426(0.40)	116(0.45)	1.02(0.82-1.26)	0.858

†Matching variables
‡ Drug prescriptions within 30 days prior to index date
Statistically significant results are highlighted in bold

4.4.2 Covariate selection

This section outlines the analysis results informing the covariate selection strategy for inclusion of covariates in the multivariable models 1 and 3 as described earlier in section 4.3.7. Tables 4.1 and Table 4.2 present the results of the association between potential confounder variables and the outcome (asthma exacerbation) and exposure variables (benzodiazepines) respectively. Table 4.3 presents results for the Model 3 covariate selection strategy whereby any covariate that modifies the unadjusted effect of the exposure of interest on the outcome by at least 10% would be included in the multivariable model. None of the covariates satisfied this strategy (Table 4.3) and therefore Model 3 was not implemented. The following confounders were significantly associated with both asthma exacerbations and benzodiazepine use and were included in Model 1:

Charlson's comorbidity index, sleep disorders, anxiety, stress, depression, psychosis, opioids, alcohol consumption, current smoking, body mass index and Index of Multiple Deprivation.

Table 4.2: Association between potential confounders and exposure to benzodiazepines (n=131,642)

Potential Confounder	**Benzodiazepines**		**Crude OR(95%CI)**	**P Value**
	No (n=110,917) %	**Yes (n=20,725)%**		
Age (years)				
<18	40073(36.13)	183(0.88)	†	†
18-44	38734(34.92)	6995(33.75)		
45-65	21958(19.80)	8837(42.64)		
>65	10152(9.15)	4710(22.73)		
Gender				
Male	50755(45.79)	5271(25.43)	†	†
Female	60162(54.24)	15454(74.57)		
IMD score in quintiles				
1 (least deprived)	14620(13.18)	2268(10.94)	1	
2	14843(13.38)	2636(12.72)	**1.14(1.05-1.23)**	P < 0.0001
3	12960(11.68)	2448(11.81)	**1.31(1.20-1.42)**	
4	13553(12.22)	2416(11.66)	**1.40(1.28-1.53)**	
5 (most deprived)	12446(11.22)	2452(11.68)	**1.76(1.60-1.94)**	
Missing	42495(38.31)	8505(41.04)	0.83(0.62-1.11)	
Current smoking				
No	66635(60.08)	14147(68.26)	1	
Yes	14990(13.51)	6483(31.28)	**1.82(1.74-1.90)**	< 0.001
Missing	29292(26.41)	95(0.46)	0.06(0.05-0.79)	
Alcohol consumption				
≤ alcohol weekly limit	29917(26.97)	9356(45.14)	1	
> alcohol weekly limit	3580(3.23)	1316(6.35)	**1.22(1.13-1.32)**	< 0.001
Missing	77420(69.80)	10053(48.51)	0.92(0.88-0.96)	
Sleep problems				
No	105616(95.22)	17178(82.89)	1	
Yes	5301(4.78)	3547(17.11)	3.16(2.97-3.36)	<0.001
Anxiety				
No	110028(99.20)	19851(95.78)	1	
Yes	889(0.80)	874(4.22)	**3.82(3.37-4.32)**	<0.001
Stress				
No	110541(99.66)	20468(98.76)	1	
Yes	376(0.34)	257(1.24)	**2.59(2.11-3.18)**	<0.001
Depression				
No	101240(91.28)	13266(64.01)	1	
Yes	9677(8.72)	7459(35.99)	**3.32(3.17-3.47)**	< 0.001
Psychosis				
No	110694(99.80)	20512(99.97)	1	

Potential Confounder	Benzodiazepines		Crude OR(95%CI)	P Value
	No (n=110,917) %	Yes (n=20,725)%		
Yes	223(0.20)	213(1.03)	**2.97(2.36-3.74)**	< 0.001
Charlson's Comorbidity index score				
0	98981(89.24)	14267(68.84)	1	
1-2	9647(8.70)	4828(23.30)	**1.70(1.61-1.78)**	P < 0.0001
3-5	2080(1.88)	1403(6.77)	**1.99(1.82-2.18)**	
>5	209(0.19)	227(1.10)	**3.22(2.56-4.06)**	
BMI				
Normal weight	23146(20.87)	6600(31.85)	1	
Underweight	1672(1.51)	417(2.01)	**1.17(1.02-1.34)**	P < 0.0001
Overweight	18625(16.79)	6096(29.41)	1.04(0.99-1.09)	
Obesity	15187(13.69)	6314(30.47)	**1.21(1.15-1.27)**	
Missing	52287(47.14)	1298(6.26)	0.34(0.37-0.43)	
Corticosteroids‡				
No	107170(96.62)	20284(97.87)	1	
Yes	3747(3.38)	441(2.13)	0.95(0.84-1.07)	0.370
Opioids‡				
No	110600(99.71)	20540(99.11)	1	
Yes	317(0.29)	185(0.89)	**1.60(1.28-1.98)**	< 0.001
Beta 2-agonists‡				
No	106389(95.92)	20121(97.09)	1	
Yes	4528(4.08)	604(2.91)	0.93(0.84-1.04)	0.196
Statins‡				
No	110555 (99.67)	20545 (99.59)	1	
Yes	362(0.33)	180(0.87)	1.21(0.98-1.49)	0.070

†Matching variables
‡ Drug prescriptions within 30 days prior to index date

Statistically significant associations are highlighted in bold

Table 4.3: Association between benzodiazepines and asthma exacerbation adjusted for individual potential confounders

Confounder adjusted for	OR	95% CI
Unadjusted	**1.46**	1.40-1.52
BMI	1.38	1.32-1.43
Alcohol consumption	1.46	1.40-1.52
Current smoking	1.37	1.32-1.43
Deprivation score	1.45	1.40-1.51
Sleeping problems	1.42	1.36-1.48
Anxiety	1.45	1.39-1.51
Stress	1.46	1.40-1.51
Psychosis	1.46	1.40-1.52
Depression	1.40	1.32-1.45
Charlson's comorbidity index score	1.45	1.39-1.51
Statins	1.46	1.40-1.52
Beta-2-agonists	1.49	1.43-1.55
Opioids	1.46	1.40-1.52
Corticosteroids	1.48	1.42-1.54

Note: ±10% change in unadjusted OR = 1.31, 1.61

OR: odds ratio; CI: confidence interval

4.4.3 Benzodiazepines and occurrence of asthma exacerbation

Table 4.4 shows results for the association between drugs acting on benzodiazepine receptors (BZPs and zopiclone) and asthma exacerbation. After adjustment for all variables (Model 2), anytime exposure to benzodiazepines was statistically significantly associated with increased occurrence of asthma exacerbation (adj mOR 1.29; 95% CI 1.23-1.35; P<0.001). When only current use of benzodiazepines was considered, the point estimate increased for occurrence of asthma exacerbation (adj mOR 1.49; 95% CI 1.15-1.93; P=0.001). Similarly, current zopiclone use was associated with increased occurrence of asthma exacerbation (adj mOR 1.59; 95% CI 1.37-1.85; p<0.001). On considering individual benzodiazepines, there was an increased likelihood of occurrence of asthma exacerbation for current use of both diazepam and temazepam. However, the association between current lorazepam and asthma exacerbation lacked statistical significance. The duration of benzodiazepine use did not alter the observed association with asthma exacerbation, with both long- and short-term users at risk (Table 4.4). Statistically significant interactions were found with comorbidity burden as measured using Charlson's comorbidity index score categories. Table 4.5 shows the stratified analysis results. The

association between anytime benzodiazepine use and asthma exacerbation was stronger among asthma patients without comorbidities.

Using unadjusted risks, we estimated that over a one year follow-up period, the number needed to harm was 22 (95% CI 12-109), indicating that for every 22 asthma patients prescribed benzodiazepines annually, one additional asthma exacerbation could be expected. Moreover, in asthma patients prescribed benzodiazepines (20,725 Patients), 4.2% of all asthma exacerbations were attributable to benzodiazepines (adjusted AF 4.23; 95% CI 3.54, 4.92). An adjusted PAF of 3.39 (95% CI 2.83, 3.94) suggests that 3.4% of asthma exacerbations in all asthma patients could be reduced if use of benzodiazepines was eliminated.

Table 4.4: Association of benzodiazepines/zopiclone exposure with asthma exacerbation (n=131,642)

Exposure	**Controls (n=105,747)%**	**Cases (n=25,895)%**	**OR (95% CI)[†]**	**Adjusted models OR (95% CI)**	
				Model 1	**Model 2**
BZP					
Never	90156(85.26)	20761(80.17)	1.00	1.00	1.00
Ever	15591(14.74)	5134(19.83)	1.46(1.40-1.52)	**1.26(1.21-1.31)**	**1.29(1.23-1.35)**
BZP timing					
Non- use	90156 (85.26)	20761(80.17)	1	1.00	1.00
Current	238(0.23)	97(0.37)	1.78(1.39-2.27)	**1.51(1.18-1.94)**	**1.49(1.15-1.93)**
Recent	415(0.39)	151(0.58)	1.63(1.34-1.97)	**1.39(1.14-1.69)**	**1.36(1.11-1.66)**
Past	14938(14.13)	4886(18.87)	1.45(1.39-1.51)	**1.25(1.20-1.30)**	**1.28(1.23-1.34)**
BZP duration					
Non-use	90156(85.26)	20761(80.17)	1.00	1.00	1.00
Short-term	14453(13.67)	4786(18.48)	1.46(1.41-1.53)	**1.27(1.21-1.32)**	**1.30(1.24-1.35)**
Long-term	1138(1.08)	348(1.34)	1.37(1.21-1.56)	**1.15(1.01-1.31)**	**1.17(1.02-1.33)**
Diazepam					
Never	95806(90.60)	22479(86.81)	1.00	1.00	1.00
Ever	9941(9.40)	3416(13.19)	1.47(1.41-1.54)	**1.31(1.25-1.37)**	**1.33 (1.27-1.40)**
Diazepam timing [£]					
Current	164(0.16)	62(0.24)	1.64(1.21-2.21)	**1.38(1.01-1.87)**	**1.42(1.04-1.94)**
Recent	289(0.27)	107(0.41)	1.60(1.27-2.01)	**1.39(1.10-1.76)**	**1.35(1.06-1.71)**

Exposure	Controls (n=105,747)%	Cases (n=25,895)%	OR (95% CI)†	Adjusted models OR (95% CI)	
				Model 1	Model 2
Past	9488(8.97)	3247(12.54)	1.47(1.40-1.53)	**1.30(1.24-1.36)**	**1.33(1.27-1.40)**
Temazepam					
Never	101774(96.24)	24666(95.25)	1.00	1.00	1.00
Ever	3973(3.76)	1229(4.75)	1.23(1.15-1.32)	**1.05(0.98-1.13)**	**1.07(0.99-1.15)**
Temazepam timing £					
Current	41(0.04)	25(0.10)	2.39(1.42-4.01)	**2.13(1.25-3.62)**	**1.93(1.10-3.38)**
Recent	87(0.08)	28(0.11)	1.28(0.82-2.00)	1.09(0.69-1.70)	1.10(0.70-1.74)
Past	3845(3.64)	1176(4.54)	1.22(1.14-1.31)	1.04(0.96-1.12)	1.06(0.98-1.14)
Lorazepam					
Never	105339(99.61)	25770(99.52)	1.00	1.00	1.00
Ever	408(0.39)	125(0.48)	1.26(1.03-1.55)	1.08(0.88-1.34)	1.11(0.90-1.38)
Lorazepam timing £					
Current	9(0.01)	1(0.00)	0.48(0.06-3.81)	0.47(0.06-3.86)	0.48(0.06-3.96)
Recent	15(0.01)	8(0.03)	2.33(0.97-5.60)	1.97(0.82-4.74)	2.29(0.96-5.47)
Past	384(0.36)	116(0.45	1.24(1.00-1.54)	1.06(0.86-1.32)	1.08(1.05-1.60)
Zopiclone					
Never	99401(94.00)	23662(91.38)	1.00	1.00	1.00
Yes	6346(6.00)	2233(8.62)	1.50(1.42-1.58)	**1.26(1.19-1.33)**	**1.28(1.21-1.36)**
Zopiclone					
Non- use	99401(94.00)	23662(91.38)	1.00	1.00	1.00
Current	645(0.61)	295(1.14)	1.91(1.65-2.20)	**1.57(1.35-1.82)**	**1.59(1.37-1.85)**
Recent	364(0.34)	135(0.52)	1.60(1.30-1.96)	**1.31(1.06-1.62)**	**1.34(1.08-1.65)**
Past	5337(5.05)	1803(6.96)	1.44(1.36-1.53)	**1.22(1.14-1.29)**	**1.24(1.16-1.32)**

Note: BZP refers to benzodiazepines; statistically significant results are highlighted in bold
£reference group is non-users; † adjusted for age, sex and general practice (matching variables); Model 1 adjusted for age, sex, general practice, Charlson's comorbidity index score, sleep disorders, stress, anxiety, depression, psychosis, opioids, alcohol, current smoking, body mass index and multiple deprivation score; Model 2: Model 1 covariates, beta 2 agonists, statins and corticosteroids.

Table 4.5: Association between benzodiazepines and asthma exacerbation stratified by Charlson's comorbidity score categories

Exposure variable	Adjusted OR† stratified by Charlson's comorbidity index (CCI) score		
	CCI score 0	**CCI score 1-2**	**CCI score ≥3**
Benzodiazepines			
No	1.00	1.00	1.00
Yes	**1.36(1.29-1.43)**	**1.24(1.08-1.43)**	1.01(0.73-1.41)
Benzodiazepines ¥			
Current	**1.60(1.18-2.19)**	0.63(0.25-1.55)	1.45(0.17-12.27)
Recent	**1.45(1.13-1.85)**	1.67(0.82-3.41)	x
Past	**1.35(1.28-1.43)**	**1.25(1.08-1.44)**	1.04(0.74-1.45)

¥Reference group is 'non benzodiazepine users'
†adjusted for age, sex, general practice, sleep disorders, stress, anxiety, depression, psychosis, opioids, beta 2 agonists and corticosteroids alcohol, current smoking, body mass index and multiple deprivation score statins

Statistically significant results are highlighted in bold
[x] could not be calculated due to insufficient data

4.4.4 Benzodiazepines and mortality following asthma exacerbation

25,887 subjects with asthma exacerbation were included in this survival analysis after further checks for data anomalies (Appendix 5.1). The median follow-up time was 1.98 years (inter-quartile range (IQR): 0.87-3.48 years) after the date of asthma exacerbation diagnosis. 35 (0.14%) subjects died within 30 days of follow-up and 459 (1.77%) died over the entire follow-up period. It was not possible to look at 30-day mortality in this study as very few deaths (n=35) occurred within 30 days of a diagnosis of asthma exacerbation. Therefore the survival analysis considered all-cause mortality occurring at any point following an asthma exacerbation over the study follow-up period (median follow-up of approximately 2 years). Post hoc validation analyses for asthma specific deaths from ONS data were also not possible as only 36 deaths were coded as due to asthma during the entire study period.

4.4.4.1 Proportional hazards assumption

A visual inspection of the log-log plots (Figure 4.1) indicate that the proportional hazards (PH) assumption was generally satisfied (the curves look roughly parallel) for both BZPs (as a class and individual BZPs except lorazepam) and zopiclone. However the statistical test

suggests that the PH assumption is met for all study drugs including lorazepam as the p value is not significant (Table 4.6).

Table 4.6: Global test for proportional Hazards assumption

Drug	P value (Global test)
Benzodiazepines	0.21
Diazepam	0.62
Temazepam	0.34
Lorazepam	0.24
Zopiclone	0.56

Figure 4.1: Log-log plots examining the Cox proportional hazards assumption

Fig 4.1a: benzodiazepines (as a class)

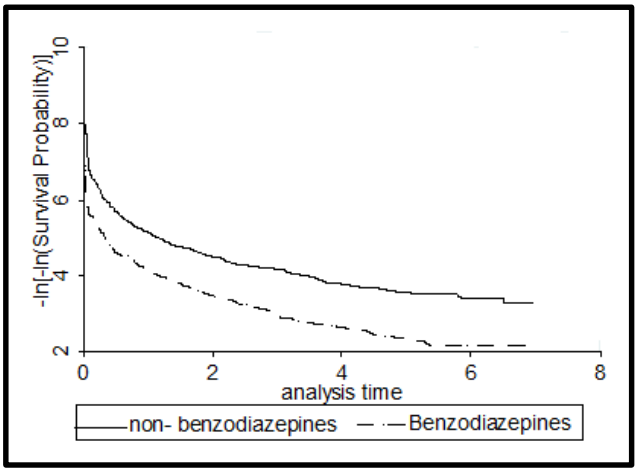


Fig 4.1b: Diazepam

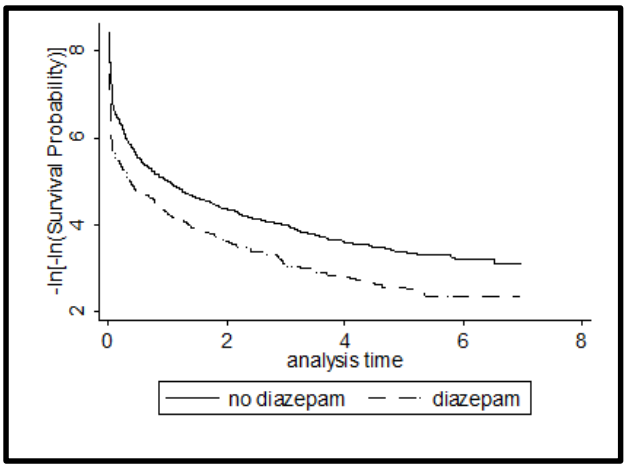


Fig4.1c: Temazepam

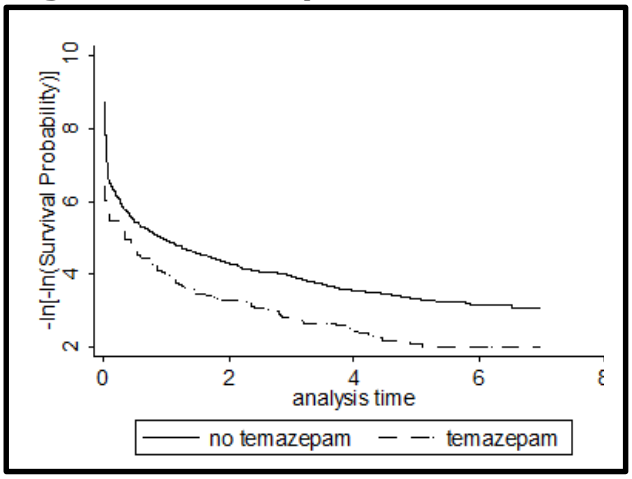
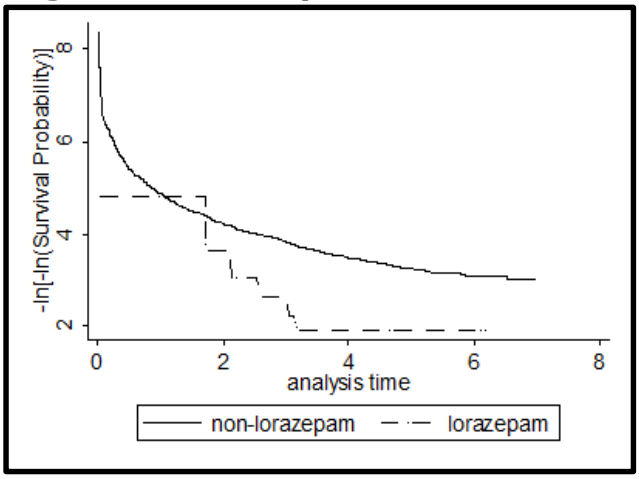
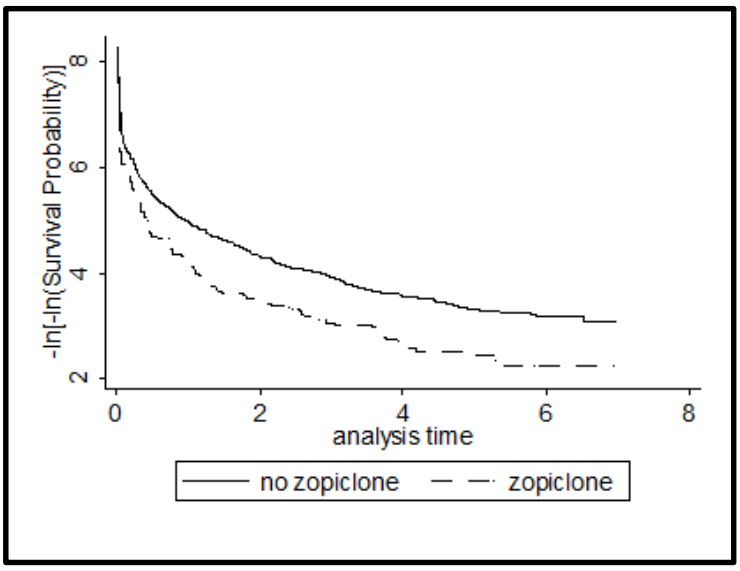


Fig 4.1d: Lorazepam



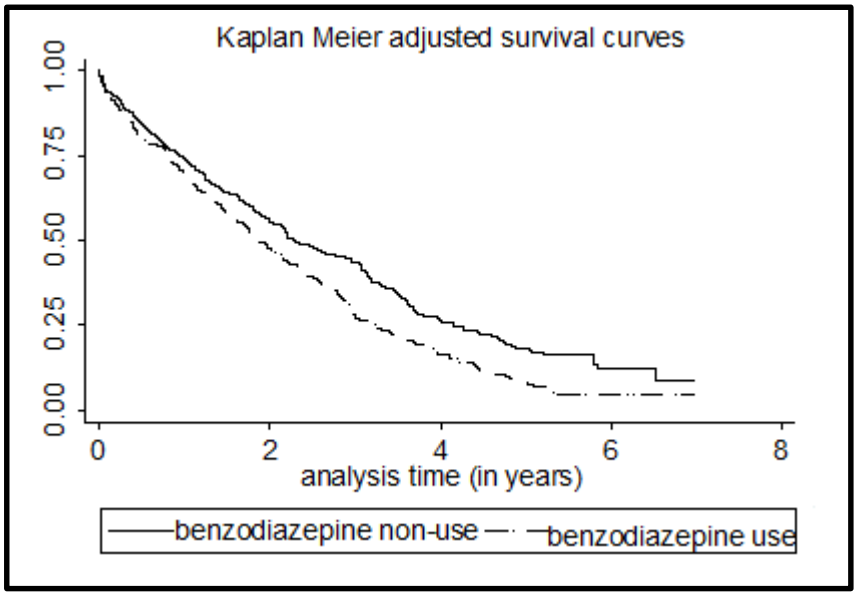


4.4.4.2 Kaplan Meier survival curves

Figure 4.2 presents the Kaplan Meier (KM) survival curves (adjusted for model 2 covariates) plotted for the entire follow-up period comparing survival in benzodiazepine users and non-users (Figure 4.2a); and zopiclone users and non-users (Figure 4.2b). The KM curves indicate increased mortality with benzodiazepine use irrespective of duration of use (Figure 4.2a). There appears to also be increased mortality with zopiclone use as compared to non-use (Figure 4.2b).

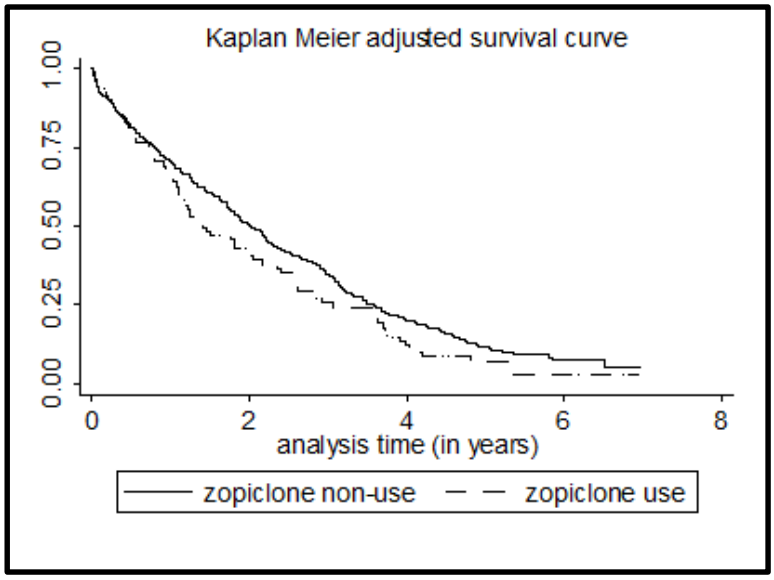
Figure 4.2: Kaplan Meier survival curves for study drug exposures (over a median follow-up period of 2 years)

Fig 4.2a: Survival following asthma exacerbation by benzodiazepines use



KM Survival curve for all-cause long term mortality subsequent to asthma exacerbation. Benzodiazepine use refers to the presence of a benzodiazepine prescription at any time before the diagnosis of asthma exacerbation. KM curves were adjusted for age, gender, Charlson's comorbidity index score, anxiety, stress, depression, psychosis, opioids, corticosteroids, beta2agonists, alcohol consumption, current smoking, body mass index and Index of Multiple Deprivation.

Fig 4.2b: Survival following asthma exacerbation by zopiclone use



KM Survival curve for all-cause long term mortality subsequent to asthma exacerbation. Zopiclone use refers to the presence of a zopiclone prescription at any time before the diagnosis of asthma exacerbation. KM curves were adjusted for age, gender, Charlson's comorbidity index score, anxiety,

stress, depression, psychosis, opioids, corticosteroids, beta2agonists, alcohol consumption, current smoking, body mass index and Index of Multiple Deprivation.

4.4.4.3 The association between benzodiazepines/zopiclone and mortality subsequent to asthma exacerbation

Table 4.7 presents HRs for the association between benzodiazepines or zopiclone exposures and all-cause mortality subsequent to asthma exacerbation over the study period (median follow-up 2 years). Benzodiazepine use was found to be significantly associated with increased mortality following asthma exacerbation (adj HR 1.32; 95% CI 1.08-1.61; p=0.006) with current and recent prescriptions associated with the greatest increase. Individually, only diazepam was significantly associated with increased mortality over the study period (adj HR 1.27; 95% CI 1.01-1.59; P=0.037). However, the number of study subjects who died (outcome of interest) was insufficient to fully investigate the effects of the other individual benzodiazepines on mortality. The unadjusted number needed to harm (NNH) was estimated as 17(95% CI 9 - 133); thus for every 17 asthma patients taking benzodiazepines, one additional death could be expected within a median follow-up time of 2 years. The association between current zopiclone use and all-cause mortality following asthma exacerbation showed borderline statistical significance (adj HR 1.61; 95% CI 0.98-2.54; P=0.058).

Table 4.7: Association between benzodiazepines/zopiclone use and all-cause mortality following asthma exacerbation in all ages (n=25,887)

Drug	Numbers dead (%) (n=459)	Unadjusted model HR(95% CI)	Adjusted models HR[1] (95% CI)	
			Model 1	**Model 2**
Benzodiazepines				
Never	272(1.31)	1.00	1.00	1.00
Ever	187(3.64)	3.14(2.61-3.79)	**1.33(1.09-1.63)**	**1.32(1.08-1.61**)
Benzodiazepines				
Non-use	246(1.31)	1.00	1.00	1.00
Current	6(7.22)	6.21(2.93-13.16)	**2.69(1.24-5.82)**	**2.78(1.26-6.12)**
Recent	6(5.30)	5.24(2.59-10.58)	**3.38(1.64-6.94)**	**3.40(1.65-6.98)**
Past	158(3.52)	3.02(2.50-3.66)	**1.27(1.04-1.56)**	**1.26(1.03-1.54)**
Diazepam				
Never	353(1.57)	1.00	1.00	1.00
Ever	106(3.10)	2.25(1.81-2.80)	**1.28(1.02-1.60)**	**1.27(1.01-1.59)**
Temazepam				
Never	403(1.63)	1.00	1.00	1.00
Ever	56(4.56)	3.02(2.28-3.40)	1.14(0.85-1.53)	1.14(0.85-1.52)
L**orazepam**				
Never	453(1.76)	1.00	1.00	1.00
Ever	6(4.80)	3.42(1.53-7.66)	1.61(0.72-3.63)	1.61(0.72-3.63)
Zopiclone				
Never	385(1.63)	1.00	1.00	1.00
Ever	74(3.31)	2.39(1.86-3.06)	1.17(0.89-1.53)	1.16(0.88-1.51)
Zopiclone timing				
Non-use	385(1.63)	1.00	1.00	1.00
Current	19(6.44)	4.58(2.89-7.27)	**1.60(1.00-2.57)**	1.58(0.98-2.54)
Recent	5(3.70)	2.86(1.18-6.92)	1.64(0.67-4.04)	1.68(0.68-4.19)
Past	50(2.77)	1.99(1.48-2.67)	1.03(0.75-1.41)	1.02(0.74-1.39)

-Model 1: Adjusted for age, gender, Charlson's comorbidity index score, sleep disorders, anxiety, stress, depression, psychosis, opioids, alcohol consumption, current smoking, body mass index and Index of Multiple Deprivation
-Model 2: Adjusted for all the factors in model 1 as well as steroids, statins and beta2agonists that failed to reach statistical significance ($p<0.05$) in univariate analysis for asthma exacerbation and benzodiazepines.
-Statistically significant results are highlighted in bold

4.4.5 Sensitivity Analyses

As benzodiazepines and other GABAergic drugs are commonly used as hypnotics and anxiolytics in adults,[143, 144] a sensitivity analysis was conducted among study subjects aged 18 years and older. The results from this sensitivity analysis for the association of benzodiazepines and asthma exacerbation were similar to original ones (sensitivity

analysis results: adj mOR 1.28; 95% CI 1.22, 1.33; P<0.001, original results: adj mOR 1.29; 95% CI 1.23, 1.35; P<0.001).

To minimise bias from misclassification of asthma exacerbation, corticosteroid prescribing was considered a proxy indication of an asthma exacerbation. A sensitivity analysis excluding controls with a current corticosteroid prescription, (1,723[1.63%]) yielded similar results to original ones (sensitivity analysis results: adj mOR 1.29; 95% CI 1.23, 1.34; P<0.001, original results: adj mOR 1.29; 95% CI 1.23, 1.35; P<0.001). In addition, an attempt was made to quantify missed exacerbations as determined from CPRD-HES linked data; 0.44% (287) of the exacerbations were recorded in HES but not in CPRD. A sensitivity analysis undertaken using only HES linked data (64,832 patients) did not affect the original results (sensitivity analysis results: adj mOR 1.25; 95% CI 1.18, 1.33; P<0.001, original analysis results: adj mOR 1.29; 95% CI 1.23, 1.35; P<0.001).

Severity of disease may influence disease outcome including all-cause mortality. However, only 0.1% (128) of the sample were recorded as having severe asthma as measured [severe asthma Read code (663V300)]. A subgroup analysis excluding these patients yielded no change in the results for the occurrence of asthma exacerbation (adj mOR 1.29; 95% CI 1.23, 1.35; P<0.001 [for both original and subgroup analysis]). In addition, hospitalisation associated with exacerbations (a proxy measure for severe asthma exacerbations) in this study, was observed in 2063 (7.97%) patients. A subgroup

analysis excluding these patients yielded comparable mortality results to original ones (subgroup analysis results: adj HR 1.40; 95% CI 1.12, 1.75; p=0.001, original results (adj HR 1.32; 95% CI 1.08, 1.61; p=0.006).

4.5 Discussion

4.5.1 Summary of main findings

The findings of this study suggest that use of benzodiazepines is associated with increased occurrence of asthma exacerbation; the heightened likelihood associated with current use increases the biological plausibility of this finding. The high prevalence of asthma as a disease and, of benzodiazepines exposure (15.7%) in this representative cohort, underscores the relevance of these findings to public health. The non-benzodiazepine drug zopiclone, which also works via activation of GABAA receptors, was associated with a similar increase in the odds of asthma exacerbation. Benzodiazepines, as well as zopiclone, were also found to be associated with increased mortality after asthma exacerbation.

4.5.2 Strengths and limitations

A review of the current literature shows that this is the first epidemiological study to examine benzodiazepine use and the occurrence of asthma exacerbation and subsequent mortality despite

the putative role of GABAA signalling in the pathogenesis of asthma. Use of the CPRD general population database has enhanced the generalisability of these findings and also allowed adequately powered analyses. Data on drug exposures are collected prospectively, prior to asthma exacerbation, thus eliminating any recall bias. Moreover, reporting bias is virtually eliminated as data are routinely collected by general practitioners during patient consultation for purposes of patient care and medico-legal record-keeping, before the conception of the research question. Using cohort data eliminates any temporal biases relating to timing of exposure. A conditional logistic regression was performed with cases and controls individually matched on age, sex, and general practice and further adjustment for a wide range of potential confounders including Charlson's comorbidity index score[125] and other specific diseases identified as confounders of the relationship between benzodiazepines and disease outcomes (anxiety, stress, psychosis and depression).[104, 126] In addition, the effect of other drugs that could be concurrently prescribed with benzodiazepines (e.g. opioids) and other factors that may trigger an asthma exacerbation (e.g. smoking) were controlled for. The data on important confounders like smoking and alcohol are usually gathered in primary care, especially for patients presenting with alcohol-related and smoking-related disease, in response to the introduction of incentive payments for better data recording in 2004 by the Quality Outcomes Framework.[145] However, the inherent

limitations of observational studies using routine databases to deal with confounding should also be recognised, in particular residual and unmeasured confounders (for example nutritional and physical factors which are not routinely recorded in CPRD). Read codes were used to identify disease variables, therefore variations in coding practices across different general practices, could have led to misclassification of asthma exacerbation. This is likely to be a non-differential bias that if it occurred, would have shifted the effect size towards unity. To minimise misclassification bias, corticosteroid prescribing was considered a proxy indication of an asthma exacerbation however this sensitivity analysis did not affect the results. Care was also taken to avoid multiple counting of a single asthma exacerbation (i.e. multiple consultations for the same episode) by analysing only the first documented asthma exacerbation in the study period.

The use of prescription data does not allow any assessment of compliance with treatment. Whilst this could lead to misclassification of exposure, the effects are likely to be non-differential and would therefore bias results towards unity. Nevertheless, the observed associations still remained when chronic benzodiazepine use as indicated by repeat prescriptions was assessed (Table 4.4).

PAF and NNH calculations suggest that benzodiazepines may account for nearly 3.4% of exacerbations in asthma patients and that one additional asthma exacerbation would be triggered within a year of

using benzodiazepines for every 22 asthma patients. In addition, the NNH findings suggest that for every 17 asthma patients taking benzodiazepines, one additional death could be expected within a median follow-up time of 2 years. However, these results should be interpreted with caution as the NNH estimates are based on unadjusted risks and while the PAF has been calculated using adjusted risk estimates, both measures assume a causal relationship that an observational study such as this cannot establish. Moreover, this study did not assess drug dosage. Future studies should look at drug dosage and duration of use in greater detail. Furthermore, it is possible that the outcomes of interest were more likely to be recorded in people who sought medical attention because they were more anxious about pre-existing comorbidities or their health in general; these individuals may be more likely to be prescribed medications for anxiety that include benzodiazepines than those who did not seek medical attention because they were generally well. While an attempt has been made to account for comorbidity burden and conditions like anxiety and stress separately, there may still be an inadvertent selection bias that cannot be fully corrected for.

4.5.3 Comparison with existing literature

It is not possible to make a direct comparison of the results from our study with earlier findings as ours is the first study we are aware of that directly assesses benzodiazepines effect in asthma patients.

Recently a similar observation has been made for patients with chronic obstructive pulmonary disease where benzodiazepines increased outpatient exacerbations and visits to the emergency room.[146] Obiora et al also found that benzodiazepines were associated with an increased risk of pneumonia and pneumonia-related mortality in a non-selected population.[126] Results for both benzodiazepines as a class and individual benzodiazepines are in line with those from the current study. In both studies, the highest risks were observed in relation to current use of benzodiazepines, particularly diazepam. The diazepam finding may be attributable to its prolonged half-life and active metabolites.[46] However, the non-significant association observed for other individual benzodiazepines with mortality could be due to the relatively low usage in the population studied and this could be a drug class effect.

It is also interesting to note that patients with a lower comorbidity burden were at increased risk of worse outcome from benzodiazepines similar to previous observations with pneumonia.[126, 147] It may be deduced that the presence of comorbidity reduces the proportion of risk attributable to benzodiazepine exposure in the individual patient.

The observation that benzodiazepine and zopiclone exposures are associated with an increased odds of asthma exacerbation is

biologically plausible given the role of GABAA receptor signalling in the pathogenesis of asthma[135] and immunity in the lung.[58] Nonetheless other studies, notably prospective cohort studies that could address some of the biases inherent in routine database studies are needed to supplement existing data on this subject.

4.5.4 Summary

Benzodiazepines are prescribed in up to one-sixth of patients with asthma. This study shows that these drugs along with zopiclone may increase the risk of asthma exacerbation; the risk of mortality after exacerbation may also be increased.

Chapter 5 : The impact of benzodiazepines on all-cause mortality in non-asthma patients: A survival analysis

5.1 Introduction to this study

This study was conducted post-hoc in response to reviewers' comments on the asthma manuscript submitted to the Lancet Respiratory Medicine journal (LRMJ [Appendix 8.0: LRMJ reviewers' comments]). The reviewer felt that a study of benzodiazepines effects on mortality in the non-asthma population would shed more light on possible pathways via which benzodiazepines influenced asthma outcomes. The reviewer's argument was that an observation of increased mortality with benzodiazepine treatment in both the asthmatic and non-asthma population would suggest that the mechanism of increased death rate is not related to GABAergic signalling in asthma. However the author of this thesis contested this assumption. The reviewer's view was not supported by the evidence in the available literature (as the impairment of immune response by GABAergic signalling is not limited to asthma). Indeed GABAergic signalling is critical in the endogenous pulmonary immune response in murine studies without asthma[58] and an important effect in an unselected population has already been demonstrated by Obiora et al.[126] Nonetheless it was agreed on discussion with the supervisory team to assess the effects of benzodiazepines effects in a population without asthma for a more holistic view on these drugs' related effect

on mortality. This change in the PhD research plan was approved by the internal examiner (Appendix 9.0).

5.2 Background

Approximately 10-20% of the global population use hypnotics, particularly benzodiazepines and Z-drugs for the treatment of anxiety and insomnia, largely because of their safety profile in comparison to drugs with similar effects for instance barbiturates.[41, 148]Other indications for these drugs include epilepsy, alcohol withdrawal, muscle spasms, sedation and palliative care.[41]

Accumulating evidence, both from animal[58, 74] and human studies[103, 105, 109, 114] (details presented in Chapter 2: Literature review), suggest increased risk of death with use of benzodiazepines and benzodiazepines-like drugs (the Z-drugs). In a recent general population study, Weich et al[104] found that exposure to hypnotics (benzodiazepines and Z-drugs) was associated with increased all-cause mortality. From animal models of infection, it has been suggested that augmented GABAergic signalling, such as through benzodiazepine treatment, impairs immune responses through intracellular acidosis of monocytes and macrophages, leading to increased mortality[58, 83] (details in Chapter 2: Literature review). In addition, benzodiazepines have also been associated with increased risk of dementia,[72] cancer[105], falls[69, 149] and road traffic accidents,[150]

which contribute to concerns for increased mortality with use of these drugs.

However, until recently, most of the evidence for the mortality effects of benzodiazepines has been from small studies which also greatly varied methodologically especially on confounder control and exposure definition.[106, 107, 113, 151] In addition, a few studies did not establish a relationship between benzodiazepines and increased mortality.[107, 152]

Recent laboratory studies have implicated benzodiazepines in the pathogenesis of asthma (details in Chapter 4: Asthma study). This study attempted to uncover whether the benzodiazepines only increase the risk of mortality in asthma or if a similar increase in mortality occurs in individuals without asthma. Thus, the objective of this study was to investigate the association between benzodiazepines and mortality in a cohort of non-asthma patients. Zopiclone, a non-benzodiazepine drug with a similar mechanism of action, was also assessed to test the possible mechanism of action underlying the observed association between benzodiazepines and mortality.

5.3 Methods

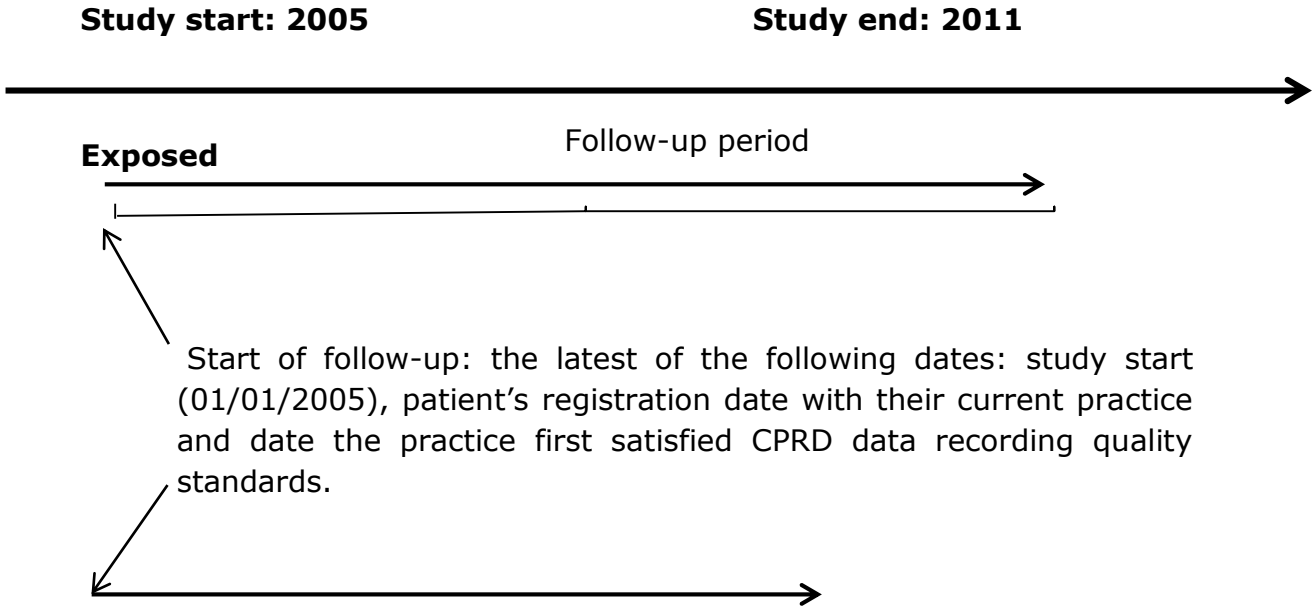
5.3.1 Data source

Data were extracted from the Clinical Practice Research Datalink (CPRD), as discussed in the General Methods Chapter (Chapter 3). CPRD linkages to the national death register (the Office of National Statistics [ONS]) were also utilised in this study.

5.3.2 Study design and population

A prospective cohort study was undertaken using CPRD to determine the association between benzodiazepine use and all-cause mortality in asthma-free patients. The study included patients of all ages (n=202,422) registered for at least 12 months in the general practice and without asthma in the study period (2005-2011).

Diagrammatic representation of the survival analysis cohort



Observations were censored if study subject transferred out of practice (lost to follow-up) or the last date CPRD collected data from the practice of their registration was before the end of study follow-up period.

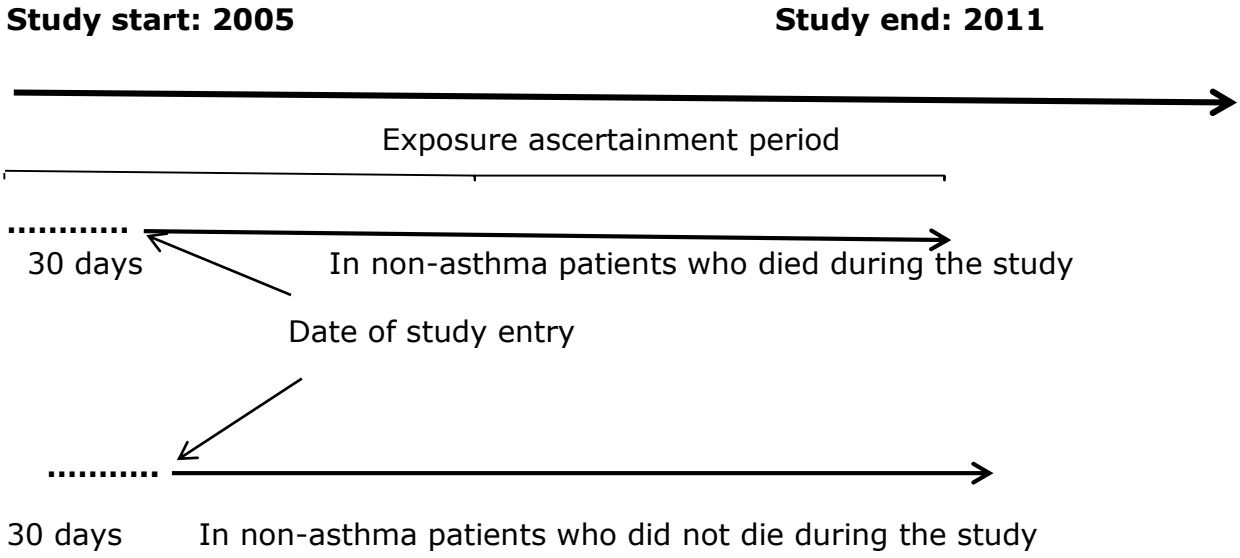
5.3.3 Outcome definition

The outcome of interest was all-cause mortality at any point during the study period (2005-2011). For study subjects with death records in both CPRD and ONS mortality data, the date of death was preferentially determined from ONS data. Death data for the remaining subjects was obtained solely from CPRD; especially for subjects not registered in English practices given that ONS provides data only for England (details in Chapter 3:General Methods).

5.3.4 Exposure definition

Exposure to benzodiazepines/zopiclone was determined at study entry as either having a prescription during the study period and/or within 30 days before study entry (to cater for current users of these drugs at the point of entry into the study). Absence of a benzodiazepine/zopiclone prescription during this period defined the unexposed study subjects. For study subjects with two or more prescriptions, a mean interval between prescription refills of less than or equal to 30 days signified continuous use and refill intervals greater than 30 days were classified as intermittent use. Patients with a single prescription were considered to have a one-off exposure to the drug.

Diagrammatic representation of exposure ascertainment



5.3.5 Study covariates

Data were extracted for various confounders as detailed in chapter 2. These included comorbidity burden as measured by Charlson's index, in addition to psychosis, depression, anxiety and depression; conditions for which benzodiazepines/zopiclone may be indicated to account for confounding by indication. Furthermore, current use of opioids, corticosteroids and statins at study entry and/or during the study period was also considered. Other potential confounders considered included age, sex, current smoking, alcohol consumption, body mass index and deprivation (the fifth quintile being most deprived and the first quintile being least deprived).

5.3.6 Statistical analyses

Cox proportional hazards modelling was used to assess the effect of benzodiazepines/zopiclone on mortality risk, adjusting for multiple confounders (as described above). Results are presented as hazard ratios (HR), with accompanying 95% confidence intervals (CIs).

Validity of the proportional hazards (PH) assumption was tested using log-log plots and the global test for proportional hazards. Kaplan-Meier survival curves were plotted over the entire study period for each of the study drugs by exposure status. All data handling and analysis was done using Stata v12 SE (Statacorp, Texas 77845 USA).

5.4 Results

After excluding patients with anomalous data, 202,422 study subjects were included in the analysis (Appendix 5.2: Patient selection flow chart). Of these, 111,062 (54.87%) were females. The median age at entry into the study was 32 years (IQR; 12-51 years). 4,926 (2.43%) subjects died during the study period. The median follow-up time was 7.00 years (IQR; 4.10-7.00 years). Benzodiazepines exposure (ever) was noted in 18,659 (9.22%) study subjects. Of the 9,404 (50.38%) benzodiazepine users with two or more prescriptions, 2,437 (25.92%) were classified as continuous users based on our study definition (section 5.3.4). Study subjects prescribed benzodiazepines were more likely to be elderly, female, to have

comorbid diseases and to be concurrent users of other drugs as compared to non-users of benzodiazepines (Table 5.1).

Table 5.1: Characteristics of exposed (benzodiazepine users) and unexposed (benzodiazepine non-users) subjects (n=202,422)

Characteristics	Benzodiazepines exposure		P value‡
	No (n=183,763)%	Yes (n=18,659)%	
Age (years)			
<18	**63206(34.40)**	**607(3.23)**	
18-44	**68142(37.08)**	**8063(43.21)**	
45-65	**35614(19.38)**	**6245(33.47)**	
>65	**16801(9.14)**	**3744(20.07)**	**<0.001**
Gender			
Male	**85677(46.62)**	**5683(30.46)**	
Female	**98086(53.38)**	**12976(69.54)**	**<0.001**
IMD score in quintiles			
1 (least deprived)	**24757(13.47)**	**2191(11.74)**	
2	**25375(13.81)**	**2397(12.85)**	
3	**22327(12.15)**	**2149(11.52)**	
4	**21998(11.97)**	**2122(11.37)**	
5 (most deprived)	**18983(10.33)**	**1769(9.48)**	**P trend <0.001**
Missing	70323(38.27)	2935(43.04)	
Current smoking			
No	90579(49.29)	12300(65.92)	
Yes	**24072(13.10)**	**5043(27.03)**	**<0.001**
Missing	69112(37.61)	1316(7.05)	
Alcohol consumption			
≤ alcohol weekly limit	**35317(19.22)**	**5500(29.48)**	
> alcohol weekly limit	**5974(3.25)**	**1324(7.10)**	**<0.001**
Missing	142472(77.53)	11835(63.43)	
Sleep problems			
No	180123(98.02)	15655(83.90)	
Yes	3640(1.98)	3004(16.10)	**<0.001**
Anxiety			
No	178607(97.19)	15247(81.71)	
Yes	**5156(2.81)**	**3412(18.29)**	**<0.001**
Stress			
No	181376(98.70)	17509(93.84)	
Yes	**2387(1.30)**	**1150(6.16)**	**<0.001**
Depression			
No	178227(96.99)	16091(86.24)	
Yes	**5536(3.01)**	**2568(13.76)**	**<0.001**
Psychosis			
No	183661(99.94)	18593(99.65)	
Yes	**102(0.06)**	**66(0.35)**	**<0.001**
Charlson's Comorbidity index score			

Characteristics	Benzodiazepines exposure		P value‡
	No (n=183,763)%	Yes (n=18,659)%	
0	156925(85.40)	12283(65.83)	
1-2	**19550(10.64)**	**4144(22.21)**	
3-5	**6352(3.46)**	**1782(9.55)**	
>5	**936(0.51)**	**450(2.41)**	**P trend <0.001**
BMI			
Normal weight	**31742(17.27)**	**4942(26.49)**	
Underweight	**2368(1.29)**	**464(2.49)**	
Over-weight	**26965(14.67)**	**4665(25.00)**	
Obesity	**20015(10.89)**	**4072(21.82)**	**P trend <0.001**
Missing	102673(55.87)	4516(24.20)	
Corticosteroids			
No	178341(97.05)	17710(94.91)	
Yes	**5422(2.95)**	**949(5.09)**	**<0.001**
Opioids			
No	168331(91.60)	11570(62.01)	
Yes	**15432(8.40)**	**7089(37.99)**	**<0.001**
Statins			
No	164465(89.50)	14092(75.52)	
Yes	**19298(10.50)**	**4567(24.48)**	**<0.001**

- ‡ p value from chi square test
- In bold are statistically significant differences between benzodiazepine exposed and unexposed study subjects with respect to given covariates.

5.4.1 Proportional hazards assumption

A visual inspection of the log-log plots (Figures 5.1a to 5.1e) suggests that the Cox PH assumption was only satisfied along the last three quarters of the follow-up time (the survival curves for benzodiazepine users and non-users seem parallel), but not from the start of follow-up. This violation of the PH assumption was confirmed by the findings from the global statistical test except in the case of temazepam (Table 5.2). This could imply that the exposure variable has different effects at different time-points. Hence, the extended Cox model was used instead of the standard Cox regression model to test the effects of benzodiazepine exposure at different time periods, i.e. effects in the first two years of follow-up and longer-term effects after 2 years. However, no significant difference in the mortality hazard ratios for the two time periods was observed (as the 95 %CI were overlapping)

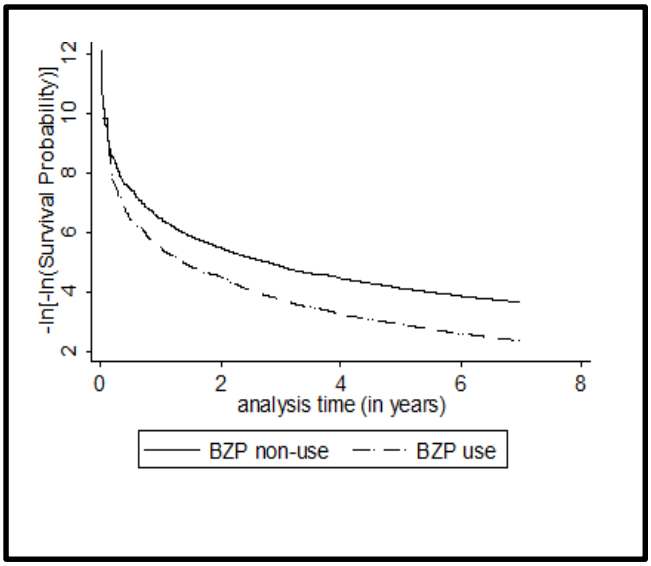
(Table 5.3) and therefore, only a single estimate (for the total follow-up period of 7 years) has been presented in a subsequent Table 5.4 which was obtained using a standard Cox regression model.

Table 5.2: Global test for proportional Hazards assumption

Drug	P value (Global test)
Benzodiazepines	0.001
Diazepam	0.001
Temazepam	0.832
Lorazepam	0.052
Zopiclone	0.001

Figure 5.1: Log-log plots examining Cox proportional hazards assumption

Fig. 5.1a) Benzodiazepines (as a class) Fig. 5.1b) Diazepam



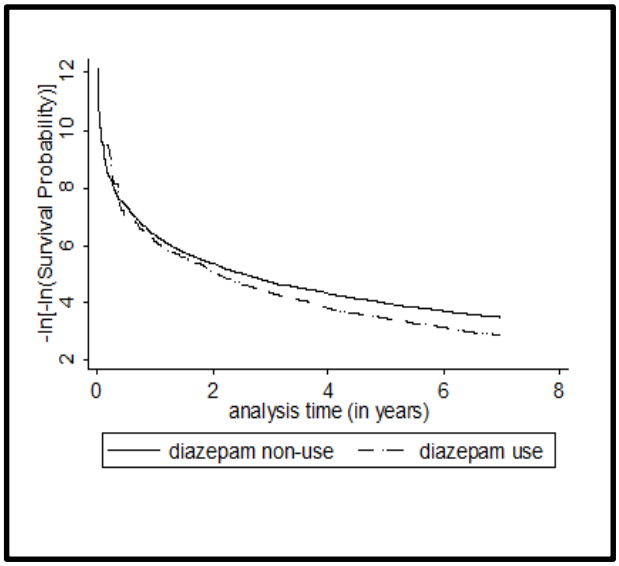


Fig. 5.1c) Temazepam

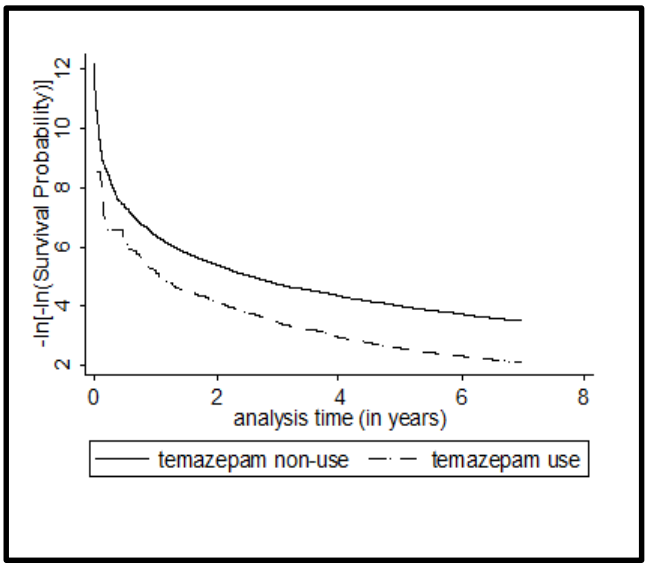


Fig. 5.1d) Lorazepam

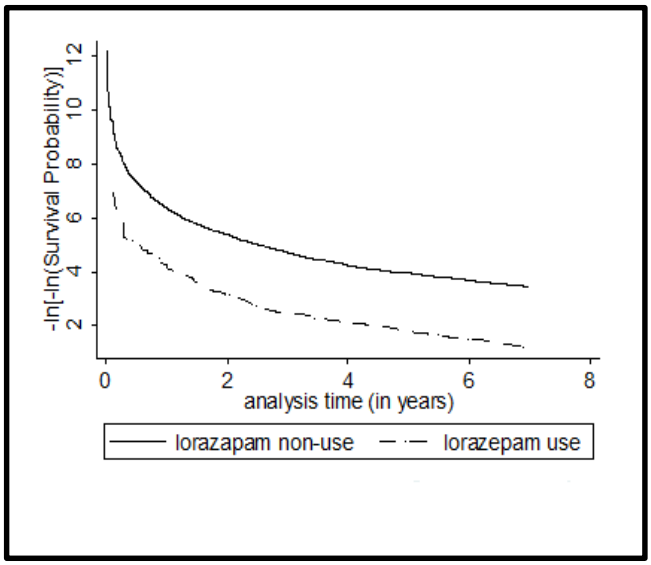
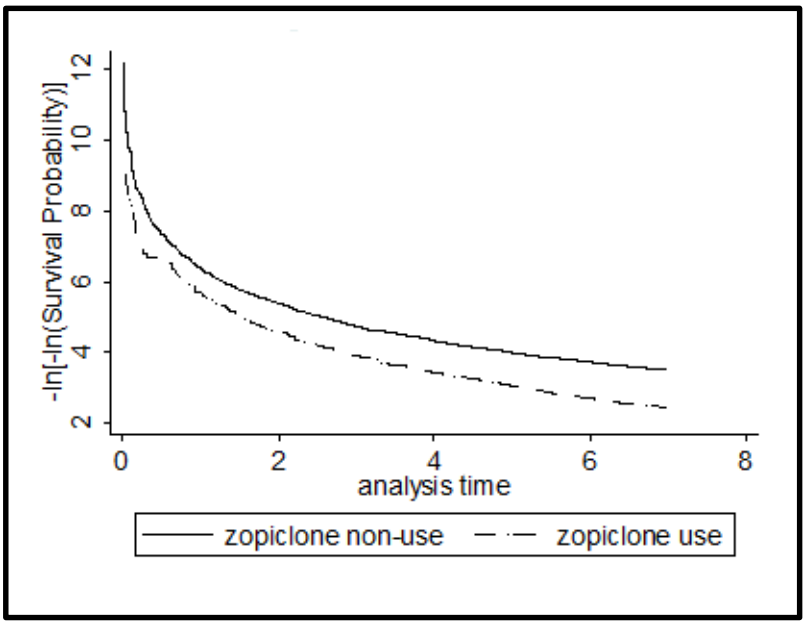


Fig 5.1e) Zopiclone

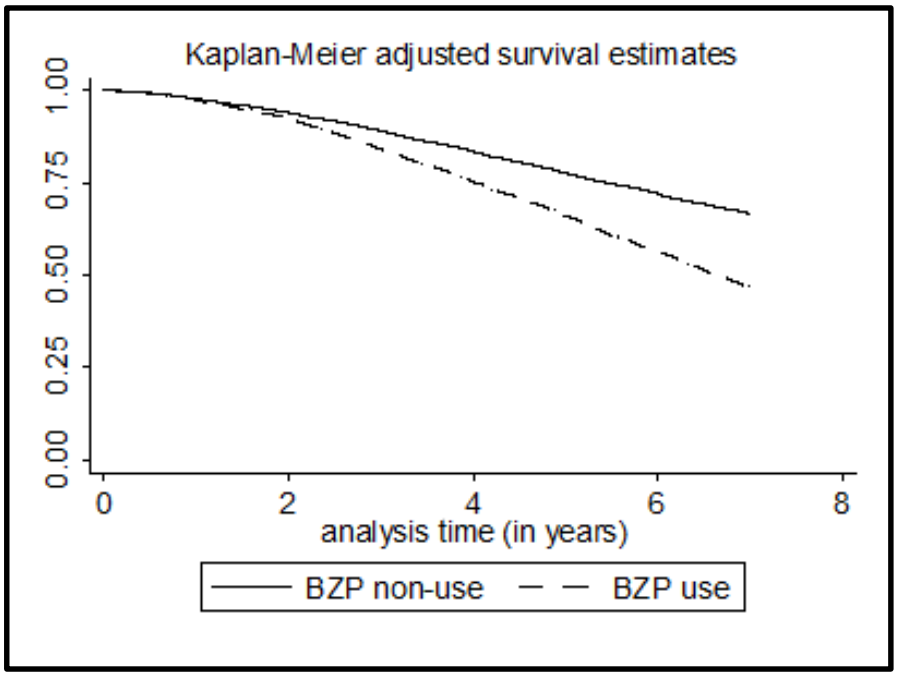


5.4.2 Kaplan Meier Survival curves

Figure 5.2 presents the adjusted Kaplan Meier (KM) survival curves (adjusted for Model 2 covariates and plotted for the entire follow-up period) comparing survival in benzodiazepine users and non-users (Figure 5.2a); and zopiclone users and non-users (Figure 5.2b). The adjusted KM curves indicate increased mortality with benzodiazepine use as well as zopiclone use.

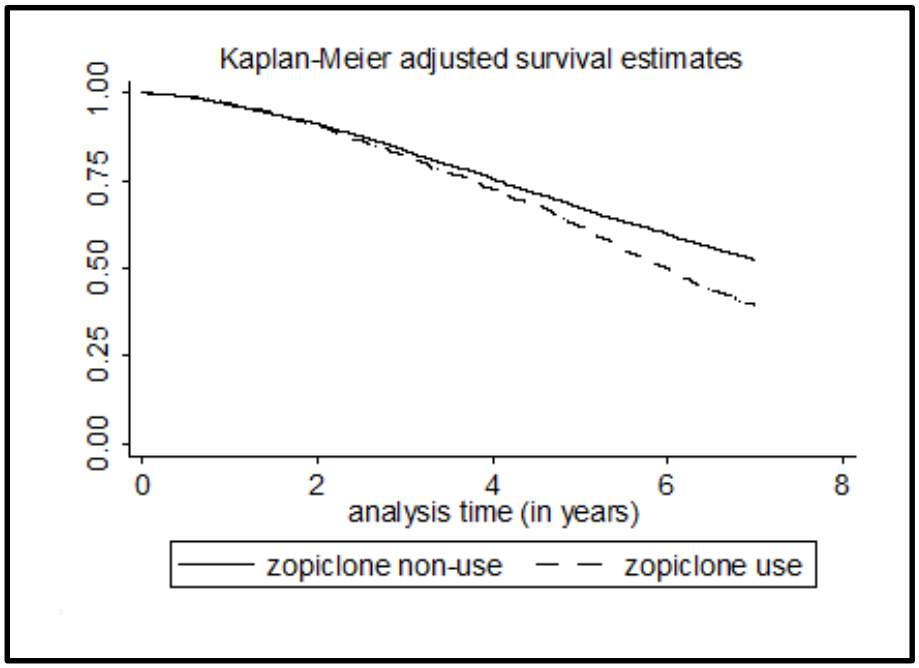
Figure 5.2: Kaplan Meier survival curves for study drug exposures (over a median follow-up of 7 years)

Fig 5.2a) Survival stratified by benzodiazepines use



Survival curves for all-cause long term mortality comparing benzodiazepines users and non-users over the entire study period (2005-2011). These curves were adjusted for age, gender, Charlson's comorbidity index score, anxiety, stress, depression, psychosis, opioids, alcohol consumption, current smoking, body mass index and Index of Multiple Deprivation

Fig 5.2b) Survival stratified by zopiclone use



Survival curves for all-cause long term mortality comparing zopiclone users and non-users over the entire study period (2005-2011). These curves were adjusted for age, gender, Charlson's comorbidity index score, anxiety, stress, depression, psychosis, opioids, alcohol consumption, current smoking, body mass index and Index of Multiple Deprivation.

Table 5.3: Mortality hazard ratios assessed for the period before and after two-years of follow-up

Drug[†]	**Unadjusted HR (95% CI)**	**Adjusted HR(95% CI)**	
		Model 1	**Model 2**
Benzodiazepines			
Follow-up <2 years	7.04(6.02-8.23)	1.72(1.44-2.06)	1.72(1.44-2.06)
Follow-up 2-7 years	3.76(3.52-4.02)	1.67(1.55-1.80)	1.66(1.54-1.79)
Diazepam			
Follow-up <2 years	3.82(3.03-4.82)	1.58(1.23-2.02)	1.58(1.23-2.04)
Follow-up 2-7 years	1.94(1.77-2.12)	1.12(1.02-1.23)	1.13(1.03-1.24)
Temazepam			
Follow-up <2 years	8.65(6.87-10.90)	1.97(1.49-2.45)	1.91(1.49-2.45)
Follow-up 2-7 years	4.10(3.71-4.53)	1.39(1.25-1.55)	1.40(1.25-1.55)
Lorazepam			
Follow-up <2 years	12.98(9.56-17-64)	1.95(1.42-2.69)	1.95(1.41-2.69)
Follow-up 2-7 years	9.70(8.40-11.18)	2.59(2.24-3.00)	2.49(2.15-2.88)
Zopiclone			
Follow-up <2 years	4.98(3.97-6.24)	1.30(1.02-1.66)	1.30(1.02-1.66)
Follow-up 2-7 years	2.93(2.67-3.22)	1.28(1.16-1.41)	1.28(1.16-1.41)

[†] Comparison group is the particular study drug non-user category
-Model 1: Adjusted for age, gender, Charlson's comorbidity index score, sleep disorders, anxiety, stress, depression, psychosis, opioids, alcohol consumption, current smoking, body mass index and Index of Multiple Deprivation (model 1 in the asthma study[chapter 4; section 4.2] for purposes of comparison of results for the two studies [asthma and non-asthma study)

-Model 2: Adjusted for all covariates described in section 5.3.5 above which includes model 1 variables plus statins and corticosteroids.

5.4.3 All-cause mortality

Table 5.4 presents HRs for the association between study drugs (benzodiazepines and zopiclone) and all-cause mortality over the median follow-up period of 7 years (IQR 4.10-7.00 years). These HRs were obtained using the standard Cox model as there were no differences in HRs obtained for the two time periods (< 2 years and 2-7 years) as established above (section 5.4.2). Benzodiazepines and zopiclone were both found to be significantly associated with increased all-cause mortality (benzodiazepines: adj HR 1.63; 95% CI 1.52-1.75; p <0.001, zopiclone: adj HR 1.29; 95% CI1.18-1.41). Hazard ratios were largest for continuous use of these drugs (benzodiazepines: adj HR 2.64; 95% CI 2.38-2.93; p <0.001, zopiclone: adj HR 1.53; 95% CI 1.32-1.79).

Table 5.4: Association between benzodiazepines/zopiclone use and all-cause mortality among non-asthmatics in all ages (median follow-up period of 7 years, IQR 4.10-7 years [n= 202,422])

Drug‡	**Number dead (%) (n=4,926)**	**Unadjusted model HR(95% CI)**	**Adjusted models HR[1] (95% CI)**	
			Model 1	**Model 2**
Benzodiazepines				
Non-use	3481(1.89)	1.00	1.00	1.00
User	1445(7.74)	3.57(3.36-3.80)	**1.64(1.53-1.76)**	**1.63(1.52-1.75)**
Benzodiazepines				
One-off use	473(5.11)	2.36(2.14-2.60)	**1.39(1.26-1.54)**	**1.38(1.25-1.53)**
Continuous use	446(18.30)	9.33(8.45-10.30)	**2.66(2.40-2.96)**	**2.64(2.38-2.93)**
Intermittent use	526(7.55)	3.37(3.07-3.69)	**1.43(1.30-1.58)**	**1.43(1.30-1.58)**
Diazepam				
Non-use	4277(2.26)	1.00	1.00	1.00
User	649(4.83)	1.82(1.68-1.98)	**1.11(1.02-1.21)**	**1.12(1.02-1.22)**

Drug‡	Number dead (%) (n=4,926)	Unadjusted model HR(95% CI)	Adjusted models HR[1] (95% CI)	
			Model 1	**Model 2**
Diazepam				
One-off use	287(3.80)	1.44(1.28-1.63)	0.98(0.87-1.11)	0.99(0.87-1.12)
Continuous use	143(9.43)	3.82(3.24-4.52)	**1.69(1.42-2.00)**	**1.70(1.44-2.02)**
Intermittent use	219(5.01)	1.83(1.60-2.10)	1.06(0.92-1.22)	1.07(0.93-1.23)
Temazepam				
Non-use	4418(2.24)		1.00	1.00
Use	508(10.09)	4.02(3.67-4.41)	**1.42(1.29-1.57)**	**1.43(1.29-1.58)**
Temazepam				
One-off use	163(6.37)	2.53(2.16-2.95)	**1.29(1.10-1.52)**	**1.29(1.10-1.51)**
Continuous use	164(21.66)	9.43(8.06-11.01)	**2.18(1.86-2.56)**	**2.19(1.86-2.57)**
Intermittent use	181(10.54)	4.07(3.51-4.73)	**1.16(1.00-1.36)**	**1.17(1.01-1.37)**
L**orazepam**				
Non-use	4685(2.33)	1.00	1.00	1.00
Use	241(23.06)	9.66(8.49-10.99)	**2.57(2.26-2.94)**	**2.49(2.18-2.84)**
Lorazepam				
One-off use	125(26.77)	11.25(9.42-13.43)	**3.14(2.62-3.76)**	**3.00(2.50-3.59)**
Continuous use	62(26.72)	11.53(8.97-14.81)	**2.35(1.82-3.03)**	**2.24(1.74-2.89)**
Intermittent use	54(15.61)	6.38(4.88-8.35)	**1.97(1.51-2.58)**	**1.97(1.51-2.58)**
Zopiclone				
Non-use	4339(2.23)	1.00	1.00	1.00
User	587(7.11)	2.82(2.59-3.08)	**1.29(1.17-1.41)**	**1.29(1.18-1.41)**
Zopiclone				
One-off use	196(4.92)	1.94(1.68-2.24)	**1.24(1.07-1.43)**	**1.23(1.07-1.43)**
Continuous use	186(14.54)	6.24(5.39-7.22)	**1.52(1.31-1.77)**	**1.53(1.32-1.79)**
Intermittent use	205(6.84)	2.66(2.31-3.06)	**1.17(1.01-1.35)**	**1.18(1.02-1.36)**

- ‡ Reference group is non-drug use
- Model 1: Adjusted for age, gender, Charlson's comorbidity index score, sleep disorders, anxiety, stress, depression, psychosis, opioids, alcohol consumption, current smoking, body mass index and Index of Multiple Deprivation (model 1 in the asthma study[chapter 4] for purposes of comparison of results from the two studies [asthma and non-asthma study)
- Model 2: Adjusted for all the potential confounders described in section 5.3.5 above which includes model 1 variables plus statins and corticosteroids.
- statistically significant results are highlighted in bold

5.4.4 Sensitivity analysis

As benzodiazepines and other GABAergic drugs are commonly used as hypnotics and anxiolytics in adults[143], a sensitivity analysis was conducted among study subjects aged 18 years and older. Results from this sensitivity analysis for the association of benzodiazepines and all-cause mortality were similar to original ones (sensitivity analysis results: adj HR 1.60; 95% CI 1.49, 1.71; P<0.001, original results: adj HR 1.63; 95% CI 1.52, 1.75; P<0.001).

5.5 Discussion

5.5.1 Summary of main findings

Benzodiazepine use, both as a class and as individual drugs (namely; diazepam, lorazepam and temazepam), was found to be associated with increased mortality after adjusting for a number of potential confounders, including physical and psychiatric comorbidities, and concurrent use of other drugs. As a class, benzodiazepines showed a nearly two-fold increase in mortality and a three-fold increase for continuous use of these drugs. A similar increase in mortality was observed with zopiclone use, a short-acting benzodiazepine agonist. Interestingly, one-off use of these drugs was also associated with increased mortality after adjustment for potential confounders.

5.5.2 Strengths and limitations

The main strength of this study was the large study sample size (n=202,422) that was sufficiently powered to detect potential adverse effects. Using the CPRD database allowed access to data on various aspects of study subjects including their medical and prescription history as well as lifestyle factors for instance, smoking and alcohol consumption. As these data are routinely collected by general practitioners during patient consultations for purposes of patient care, reporting bias was virtually eliminated. We also took advantage of CPRD linkages to the ONS national death registry in the England, to validate the outcome death initially ascertained from

CPRD data only. We controlled for a wide range of physical and psychiatric conditions (including depression, psychosis and anxiety) as this is especially important given the possibility of confounding by indication. Furthermore, the effect of drugs that could be concurrently prescribed with benzodiazepines (e.g. opioids and steroids) and other factors known to influence survival were controlled for.

However, this is an observational study that used a routine database (CPRD), and therefore, the inherent limitations relating to confounding should be recognised, particularly, residual and unmeasured confounders (for example nutritional and physical factors which are not routinely recorded in CPRD). In addition, drug prescription does not mean use of the drug therefore misclassification of exposure status may have occurred but at most, this is likely to lead to a non-differential bias and shift the results towards unity.

Immortal time bias may be a problem in cohort studies as study subjects who survive longer would be more likely to get exposed to study drugs than those who die earlier in the study. To account for this an extended Cox regression model was employed to account for time varying effects of benzodiazepine exposure but no statistically significant difference was observed between the mortality hazard ratios for the initial and later part of the study follow-up period.

5.5.3 Comparison with existing literature

Our observation of increased mortality with benzodiazepines use is in line with findings from previous studies[103, 109, 111, 126, 153] particularly the most recent studies undertaken in the general population.[104, 105] Over an average follow-up period of 2.5 years of 34,205 patients from the general population, Kripke et al reported an over four-fold increase in mortality with hypnotic use (including benzodiazepines).[105] Weich et al also established a heightened mortality risk with hypnotic use from their study of 104,145 patients in CPRD over a mean follow-up of 7.6 years[104], although at a slightly lower estimate of three-fold increased mortality but which is comparable to results from the current study that has a similar study follow-up period (median follow-up 7.00 years; IQR 4.10-7.00 years). The differences in the effect sizes may be explained by variations in the length of follow-up period as both Kripke et al and Weich et al found a declining association between hypnotics and increased mortality over time.[104, 105]

Unlike most studies, only the present study and the study by Kripke et al[105] assessed the association between brief exposures to hypnotics (single prescription in the current study and <18 pills in a year in Kripke et al's study) and mortality. Surprising, in both studies an increased risk of death was established even following a brief exposure to benzodiazepines (after adjusting for various confounders). Notably, the effect estimates for one-off or brief exposures to hypnotics was weaker than those obtained for

continuous use of these drugs; this is in keeping with the biological plausibility of their effects (i.e. constant activation of the GABAA receptors from continuous use of GABAergic drugs may be assumed to have a greater impact on the body's immune system than would be expected from brief exposures to such drugs).

5.5.4 Summary

This large population study shows an increased risk of mortality in non-asthmatics with the use of benzodiazepines as well as zopiclone use. Continuous use of these drugs is associated with greater mortality effects. These findings are in line with reports from previous cohort studies.

Chapter 6 : The association between benzodiazepines and influenza-related outcomes (pneumonia and mortality): A survival analysis

6.1 Introduction to this chapter

In this chapter, a brief overview of literature is first presented as the background to the study (the detailed literature review is presented in Chapter 2). This is followed by the study methods, results and discussion.

6.2 Background

As noted in Chapter 1: Section 1.3 (Introduction to ILI), influenza affects many lives and is usually complicated with bacterial superinfection particularly pneumonia.[154] In the United Kingdom, annual influenza deaths were estimated between 18500 and 25000 for the years 1996 to 2004.[30] One potentially important strategy to reduce influenza morbidity and mortality is to determine whether commonly prescribed drugs modify susceptibility to bacterial superinfection in influenza like illness (ILI) or alter subsequent mortality.

The benzodiazepine drugs have recently been suggested as having detrimental effects on immune response to infection mediated through their actions on the GABAA receptors (details in Chapter 2: Literature review).[58] In animal studies, benzodiazepines increased mortality from Klebsiella pneumoniae,[74] Mycobacterium bovis[89] and Salmonella typhimurium.[88] Sanders et al. recently showed that

diazepam increases the hazard ratio of death from Streptococcus pneumonia (HR: 7.565 95% CI 2.32-24.71; p = 0.0043) and influenza pneumonia (HR 5.857 95% CI 1.366 to 25.10; p = 0.023) in C57BL/6 mice.[58] In addition, mice treated with diazepam that survived influenza infection, had evidence of spontaneous bacterial superinfection in their lungs.[58] In a population based study, Obiora et al. reported an increased risk of pneumonia and subsequent mortality with benzodiazepine use.[126]

Given the apparent increased mortality related to influenza infection in animal studies and the increased susceptibility to bacterial superinfection attributed to benzodiazepines, the present study aimed to investigate these effects in the human population; the test hypothesis being that benzodiazepine use would increase the risk of developing ILI-related pneumonia and death in patients with influenza like illness. The same hypothesis was tested among users of zopiclone, a hypnotic drug with a similar mechanism of action as the benzodiazepines.

6.3 Methods

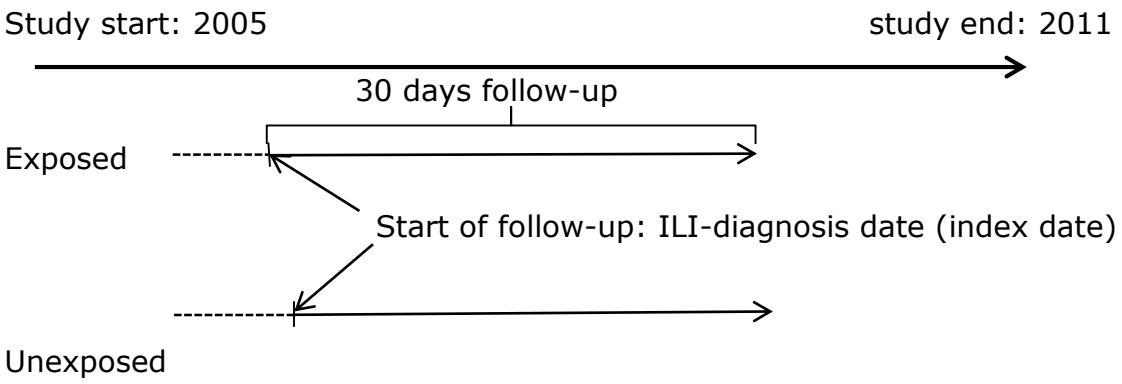
6.3.1 Data source

This study utilised data from the Clinical Practice Research Datalink (CPRD) which contains the medical records of over 13 million patients prospectively collected from over 600 general practices across the UK, and linked data from the Office of National Statistics (ONS) mortality database as described in Chapter 3 (General Methods).

6.3.2 Study design and population

A prospective cohort study was undertaken to assess the association between benzodiazepines and ILI-related pneumonia and ILI-related mortality. This study included 804,337 patients of all ages with a recorded diagnosis of influenza-like illness within the study period (2005-2011[Appendix 5.3: Patient selection flow chart]), identified using a defined set of medical Read codes (Appendix 3.2: ILI Read Codes). Patient outcomes were ascertained over a 30-day follow-up period starting at the point of ILI diagnosis. Patients who developed the outcome at the start of follow-up (i.e. day of ILI diagnosis or consultation) were also included in the analysis to overcome potential biases due to exclusion of delayed diagnoses or more severe cases.

Diagrammatic representation of the survival analysis cohort



Note: BZP exposure was ascertained before ILI (as illustrated below in section 6.3.4)

Observations were censored if patient transferred out of practice (lost to follow-up) or death occurred for the ILI-pneumonia outcome or follow-up was completed before event occurred (for both pneumonia and death outcomes).

6.3.3 Outcome definition

There were two outcomes of interest; 1) ILI-related pneumonia and 2) ILI-related mortality. ILI-related pneumonia was defined as physician diagnosed pneumonia occurring within 30 days after the diagnosis of ILI. Data on pneumonia were identified using relevant Read codes (Appendix 3.3: Pneumonia Read codes) and the first ever eligible record of pneumonia in the study period was retained in the analyses (General Methods Chapter; Table 3.4: pneumonia Read codes included in the analyses).

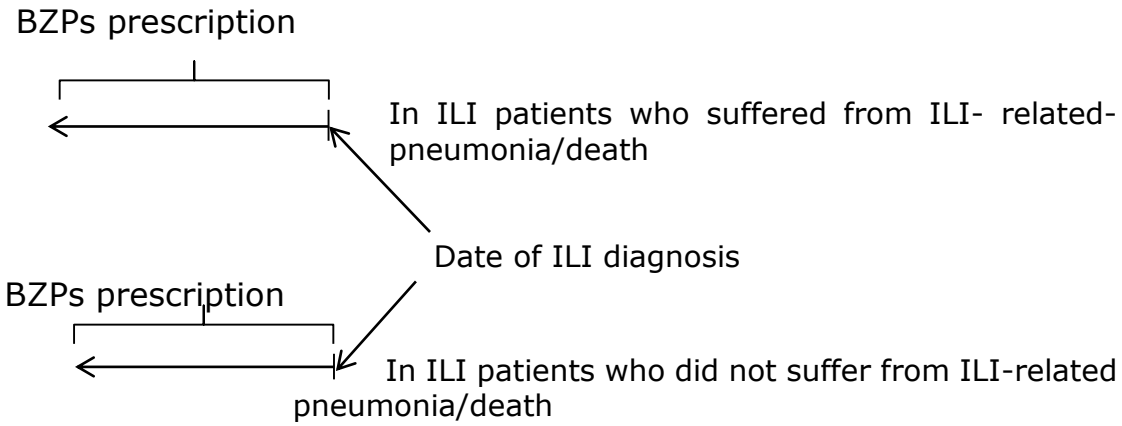
Deaths occurring within the 30 day follow-up period, regardless of cause, were considered as potentially 'ILI-related'. For patients with death records in both CPRD and ONS mortality data, the date of death was preferentially determined from ONS data. Death data for the remaining subjects were obtained solely from CPRD records (ONS mortality data are available only for subjects registered with English practices that had signed-up for CPRD-ONS linkage as previously described in the General Methods [Chapter 3]).

6.3.4 Exposure definition

Data on all recorded prescriptions of benzodiazepines/zopiclone for study patients were extracted. Benzodiazepines were considered both as a class and as individual drugs, represented by the three most frequently prescribed benzodiazepines in the study population

namely, diazepam, temazepam and lorazepam. Exposure was considered current when the most recent prescription was within 30 days prior to the ILI diagnosis date. The presence of a repeat prescription was used as a proxy indicator of long term drug use whereas the absence of it was considered short term use. Prescriptions in the period 31 to 90 days and >90 days before ILI diagnosis date were classified as recent and past exposures respectively. No evidence of prescribing at any point in the patient's past primary care records defined 'non-users'.

Diagrammatic representation of exposure ascertainment



6.3.5 Study covariates

A wide range of potential confounders were considered as discussed in the General Methods chapter (Chapter 3: Section 3.6.3). These include comorbidity burden measured by the Charlson's comorbidity index score, depression, anxiety, stress, psychosis, insomnia and

other sleep disorders (as conditions in which benzodiazepines/zopiclone may be indicated and which have been identified as important confounders in previous related studies.)[104, 126] Furthermore, current prescriptions of opioids, corticosteroids, statins, antibiotics, antivirals, influenza vaccination in the year preceding the diagnosis of ILI were considered, as well as current smoking, alcohol consumption, body mass index, deprivation, gender and age at ILI diagnosis.

6.3.6 Statistical analyses

The Cox proportional hazards regression analysis was used in both the analyses for ILI-related pneumonia and ILI-related mortality. Results are presented as unadjusted and adjusted hazard ratios (HR), with accompanying 95% confidence intervals (CIs). Two multivariable models (Model 1 and Model 2) were constructed for each exposure variable. Model 1 included all variables which were either:

a) significantly associated with both ILI-related pneumonia or ILI-related mortality and benzodiazepines ($P<0.05$); or
b) Variables that modified unadjusted effect of benzodiazepine on the outcomes of interest by at least 10%.

Model 2 included all a prior confounders as listed in section 6.3.5 above.

Validity of the proportional hazards (PH) assumption was tested using log-log plots and the global test for proportional hazards. Adjusted

survival curves were plotted for ILI-related pneumonia and mortality by exposure status for each study drug. All data handling and analysis was done in Stata v12 SE (Statacorp, Texas 77845 USA).

6.4 Results

Of the 804,337 ILI patients identified in the study period, 804,051 were included in the analyses after exclusion of those with anomalous data (Appendix 5.3: Study patient selection flow chart). The median age at ILI diagnosis was 25 years (IQR; 4 to 48 years). During the 30 days of follow-up, 1,117(0.14%) patients developed pneumonia while 707(0.09%) died. Benzodiazepines and zopiclone use was noted in 60,282 (7.5%) and 25,578 (3.18%) study patients respectively. Study patients prescribed benzodiazepines were more likely to be adults, female, users of other drugs and have comorbid diseases as compared to non-users of benzodiazepines (Table 6.1).

Table 6.1: Characteristics of exposed (benzodiazepine users) and unexposed (benzodiazepine non-users) patients (n=804,051)

Characteristics	**Benzodiazepines exposure**		**P value‡**
	No (n=743,769)%	**Yes (n=602,82)%**	
Age (years)			
<18	343128(46.13)	675(1.12)	
18-44	**218717(29.41)**	**21794(36.15)**	
45-65	**113376(15.24)**	**23498(38.98)**	
>65	**68548(9.22)**	**14315(23.75)**	**P trend <0.001**
Gender			

Characteristics	**Benzodiazepines exposure**		**P value‡**
	No (n=743,769)%	**Yes (n=602,82)%**	
Male	326670(43.92)	18455(30.61)	
Female	**417099(56.08)**	**41827(69.39)**	**<0.001**
IMD score in quintiles			
1 (least deprived)	100914(13.57)	8184(13.58)	
2	96764(13.01)	8257(13.70)	
3	77532(10.42)	6663(11.05)	
4	89771(12.07)	6960(11.55)	
5 (most deprived)	81874(11.01)	5962(9.89)	**P trend <0.001**
Missing	296914(39.92)	24256(40.24)	
Current smoking			
No	240337(32.31)	33048(54.82)	
Yes	**67022(9.01)**	**12593(20.89)**	**<0.001**
Missing	436410(58.68)	14641(24.29)	
Alcohol consumption			
≤ alcohol weekly limit	150076(20.18)	24043(39.88)	
> alcohol weekly limit	**14875(2.00)**	**2665(4.42)**	**<0.001**
Missing	578818(77.82)	33574(55.69)	
Sleep disorders			
No	715381(96.18)	52399(86.92)	
Yes	**28388(3.82)**	**7883(13.08)**	**<0.001**
Anxiety			
No	737374(99.14)	58282(96.68)	
Yes	**6395(0.86)**	**2000(3.32)**	**<0.001**
Stress			
No	741232(99.66)	59622(98.91)	
Yes	**2537(0.34)**	**660(1.09)**	**<0.001**
Depression			
No	738895(99.34)	59209(98.22)	
Yes	**4874(0.66)**	**1073(1.78)**	**<0.001**
Psychosis			
No	743683(99.99)	60269(99.98)	
Yes	**86(0.01)**	**13(0.02)**	**0.033**
Charlson's Comorbidity index score			
0	**593787(79.83)**	**35530(58.94)**	
1-2	**131932(17.74)**	**19960(33.11)**	
3-5	**15097(2.03)**	**3891(6.45)**	
>5	**2953(0.40)**	**901(1.49)**	**P trend <0.001**
BMI			
Normal weight	**108723(14.62)**	**16419(27.24)**	
Underweight	**7436(1.00)**	**946(1.57)**	
Overweight	**79443(10.68)**	**13870(23.01)**	
Obesity	**56079(7.54)**	**10995(18.24)**	**P trend < 0.001**
Missing	492088(66.16)	18052(29.95)	
Corticosteroids			

Characteristics	Benzodiazepines exposure		P value‡
	No (n=743,769)%	**Yes (n=602,82)%**	
No	736027(98.96)	59499(98.70)	
Yes	**7742(1.04)**	**783(1.30)**	**<0.001**
Opioids			
No	739100(99.37)	59657(98.96)	
Yes	**4669(0.63)**	**625(1.04)**	**<0.001**
Statins			
No	742569(99.84)	60009(99.55)	
Yes	**1200(0.16)**	**273(0.45)**	**<0.001**
Antivirals			
No	738977(99.36)	59975(99.49)	
Yes	**4792(0.64)**	**307(0.51)**	**<0.001**
Antibiotics			
No	743282(99.93)	60234(99.92)	
Yes	487(0.07)	48(0.08)	0.195
Flu vaccination			
No	739386(99.41)	59475(98.66)	
Yes	**4383(0.59)**	**807(1.34)**	**<0.001**

- ‡p-values from chi square test.
- In bold are statistically significant differences (i.e. p<0.05) between benzodiazepine users and non-users with regard to covariates.

6.4.1 Selection of covariates

This section outlines the covariates selection strategy for identification of covariates for inclusion in the multivariable model 1 as described earlier in section 6.3.6. Tables 6.1 and 6.2 present the results of the association between potential confounders and benzodiazepines and the study outcomes (ILI-related pneumonia and mortality) respectively. Table 6.3 presents results that informed the covariate selection strategy which identified covariates that led to at least a 10% change in the unadjusted effect of benzodiazepines on the respective study outcomes.

Covariates that met the inclusion criteria for Model 1 in the ILI-related pneumonia analyses were age, deprivation, current smoking, alcohol

use, sleep disorders, Charlson Comorbidity index score and BMI. Model 1 in the ILI-related mortality analyses included age, Charlson's comorbidity index score, anxiety, sleeping disorders, opioids, alcohol consumption, current smoking, body mass index, statins, antivirals and influenza vaccination.

Table 6.2: Association between potential confounders and outcomes of interest (ILI-related pneumonia and mortality [n=804,051])

Characteristics	ILI-related pneumonia		P value‡	ILI-related mortality		P value‡
	No (n=802,934)%	Yes (n=1,117)%		No (n=803,344)%	Yes (n=707)%	
Age (years)						
<18	343449(42.77)	354(31.69)		343784(42.79)	19(2.69)	
18-44	240159(29.91)	352(31.51)		240478(29.93)	33(4.67)	
45-65	136685(17.02)	189(16.92)		136789(17.03)	85(12.02)	
>65	**82641(10.29)**	**222(19.87)**	**P<0.001**	**82293(10.24)**	**570(80.62)**	**<0.001**
Gender						
Male	344628(42.92)	497(44.49)		344814(42.92)	311(43.99)	
Female	458306(57.08)	620(55.51)	0.288	458530(57.08)	396(56.01)	0.567
IMD score in quintiles						
1 (least deprived)	108975(13.57)	123(11.01)		109005(13.57)	93(13.15)	
2	104881(13.06)	140(12.53)		104922(13.06)	99(14.00)	
3	84085(10.47)	110(9.85)		84119(10.47)	76(10.75)	
4	96611(12.03)	120(10.74)		96661(12.03)	70(9.90)	
5 (most deprived)	87725(10.93)	111(9.94)	**0.002**	87764(10.92)	72(10.18)	
Missing	320657(39.94)	513(45.93)		320873(39.94)	297(42.01)	0.493
Current smoking						
No	272921(33.99)	464(41.54)		272871(33.97)	514(72.70)	
Yes	**79477(9.90)**	**138(12.35)**	**<0.001**	**79505(9.90)**	**110(15.56)**	**<0.001**
Missing	450536(56.11)	515(46.11)		450968(56.14)	83(11.74)	
Alcohol consumption						
≤ alcohol weekly limit	173842(21.65)	277(24.80)		173877(21.64)	242(34.23)	
> alcohol weekly limit	**17515(2.18)**	**25(2.24)**	**0.036**	**17513(2.18)**	**27(3.82)**	**<0.001**
Missing	611577(76.17)	815(72.96)		611954(76.18)	438(61.95)	
Sleep disorders						
No	766739(95.49)	1041(93.20)		767163(95.50)	617(87.27)	
Yes	**36195(4.51)**	**76(6.80)**	**<0.001**	**36181(4.50)**	**90(12.73)**	**<0.001**
Anxiety						
No	794550(98.96)	1106(99.02)		794965(98.96)	691(97.74)	
Yes	8384(1.04)	11(0.98)	0.845	**8379(1.04)**	**16(2.26)**	**0.001**

Characteristics	**ILI-related pneumonia**		**P value‡**	**ILI-related mortality**		**P value‡**
	No (n=802,934)%	**Yes (n=1,117)%**		**No (n=803,344)%**	**Yes (n=707)%**	
Stress						
No	799743(99.60)	1111(99.46)		800149(99.60)	705(99.72)	
Yes	3191(0.40)	6(0.54)	0.458	3195(0.40)	2(0.28)	0.628
Depression						
No	796993(99.26)	1111(99.46)		797405(99.26)	699(98.87)	
Yes	5941(0.74)	6(0.54)	0.429	5939(0.74)	8(1.13)	0.224
Psychosis						
No	802835(99.99)	1117(100.00)		803245(99.99)	707(100)	
Yes	99(0.01)	0(0.00)	0.711	99(0.01)	0(0.00)	0.768
Charlson's Comorbidity index score						
0	628559(78.28)	758(67.86)		629121(78.31)	196(27.72)	
1-2	**151618(18.88)**	**274(24.53)**		**151593(18.87)**	**299(42.29)**	
3-5	**18915(2.36)**	**73(6.54)**		**18827(2.34)**	**161(22.77)**	
>5	**3842(0.48)**	**12(1.07)**	**<0.001**	**3803(0.47)**	**51(7.21)**	**<0.001**
BMI						
Normal weight	124935(15.56)	207(18.53)		124945(15.55)	197(27.86)	
Underweight	**8363(1.04)**	**19(1.70)**		**8343(1.04)**	**39(5.52)**	
Over-weight	**93177(11.60)**	**136(12.18)**		**93171(11.60)**	**142(20.08)**	
Obesity	**66955(8.34)**	**119(10.65)**	**<0.001**	**66979(8.34)**	**95(13.44)**	**<0.001**
Missing	509504(63.46)	636(56.94)		509906(63.47)	234(33.10)	
Corticosteroids						
No	794427(98.94)	1099(98.39)		794840(98.94)	686(97.03)	
Yes	8507(1.06)	18(1.61)	0.072	**8504(1.06)**	**21(2.97)**	**<0.001**
Opioids						
No	797650(99.34)	1107(99.10)		798078(99.34)	679(96.04)	
Yes	5284(0.66)	10(0.90)	0.327	**5266(0.66)**	**28(3.96)**	**<0.001**
Statins						
No	801465(99.82)	1113(99.64)		801890(99.82)	688(97.31)	
Yes	1469(0.18)	4(0.36)	0.171	**1454(0.18)**	**19(2.69)**	**<0.001**
Antivirals						
No	797841(99.37)	1111(99.46)		798245(99.37)	707(100)	
Yes	5093(0.63)	6(0.54)	0.683	5099(0.63)	0(0.00)	0.034

Characteristics	ILI-related pneumonia		P value‡	ILI-related mortality		P value‡
	No (n=802,934)%	**Yes (n=1,117)%**		**No (n=803,344)%**	**Yes (n=707)%**	
Antibiotics						
No	802399(99.93)	1117(100.00)		802812(99.93)	704(99.58)	
Yes	535(0.07)	0(0.00)	0.388	**532(0.07)**	**3(0.42)**	**<0.001**
Flu vaccination						
No	797755(99.35)	1106(99.02)		798187(99.36)	674(95.33)	
Yes	5179(0.65)	11(0.98)	0.156	**5157(0.64)**	**33(4.67)**	**<0.001**

- ‡p-values from chi square test.
- In bold are statistically significant differences (i.e. p<0.05) between study outcomes with regard to covariates.

Table 6.3: Association between benzodiazepines and study outcomes adjusted for individual confounders

Confounder adjusted for	ILI-related pneumonia		ILI-related mortality	
	HR	**95% CI**	**HR**	**95% CI**
Unadjusted	1.72	1.43-2.05	4.42	3.74-5.22
Age	**1.40**	1.16-1.68	**1.84**	1.55-2.18
Gender	1.75	1.45-2.08	4.51	3.81-5.34
BMI	1.59	1.32-1.92	**3.17**	2.67-3.78
Alcohol consumption	1.67	1.40-2.01	**3.95**	3.32-4.68
Current smoking	**1.53**	1.27-1.83	**2.77**	2.34-3.28
Deprivation score	1.71	1.43-2.05	4.41	3.73-5.21
Sleeping problems	1.65	1.38-1.98	**3.98**	3.35-4.72
Anxiety	1.72	1.44-2.06	4.36	3.69-5.16
Stress	1.71	1.43-2.05	4.44	3.75-5.24
Psychosis	1.72	1.43-2.05	4.42	3.74-5.22
Depression	1.72	1.44-2.06	4.41	3.73-5.21
Charlson's comorbidity index score	**1.49**	1.24-1.79	**2.39**	2.01-2.84
Statins	1.71	1.43-2.05	4.29	3.63-5.07
Opioids	1.71	1.43-2.05	4.34	3.67-5.13
Corticosteroids	1.71	1.43-2.05	4.40	3.72-5.20
Antivirals	1.72	1.43-2.05	4.41	3.73-5.22
Antibiotics	1.72	1.43-2.05	4.42	3.74-5.22
Flu vaccination	1.71	1.43-2.05	4.26	3.60-5.03

Note: ±10% change in adjusted HR: ILI-related pneumonia; 1.55, 1.89, ILI-related death; 3.98, 4.86. Highlighted in bold are covariates that changed the unadjusted HR by at least ±10%

HR: Hazard ratio, CI: Confidence interval.

6.4.2 Proportional hazards assumption

A visual inspection of the log-log plots (Figure 6.1 and 6.2) indicate that the Cox PH was satisfied for both benzodiazepines and zopiclone. This was also reflected in the global test findings (Table 6.5) except for the relationship between benzodiazepines exposure and 30-day mortality (P=0.04). Even in the latter case, the mortality hazards were judged as meeting the proportionality assumption over the short term (i.e. 30 days of follow-up) based on the graphical presentation of the hazards which showed reasonably parallel lines for benzodiazepines users and non-users (Figure 6.2a). Therefore, it was

deemed appropriate to use the standard Cox regression model for all subsequent analyses.

Figure 6.1: Log-log plots examining Cox proportional hazards assumption for the association between drug use and ILI-related pneumonia

Fig. 6.1a) Benzodiazepines **Fig 6.1b) Zopiclone**

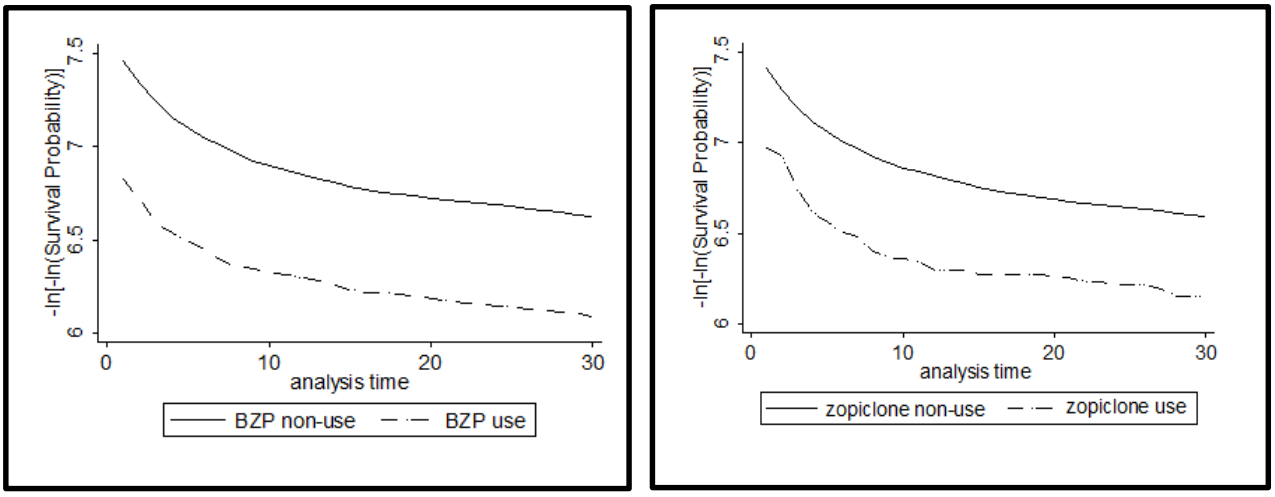


Figure 6.2: Log-log plots examining Cox proportional hazards assumption for the association between drug use and ILI-related death

Fig 6.2a) Benzodiazepines **Fig 6.2 b) Zopiclone**

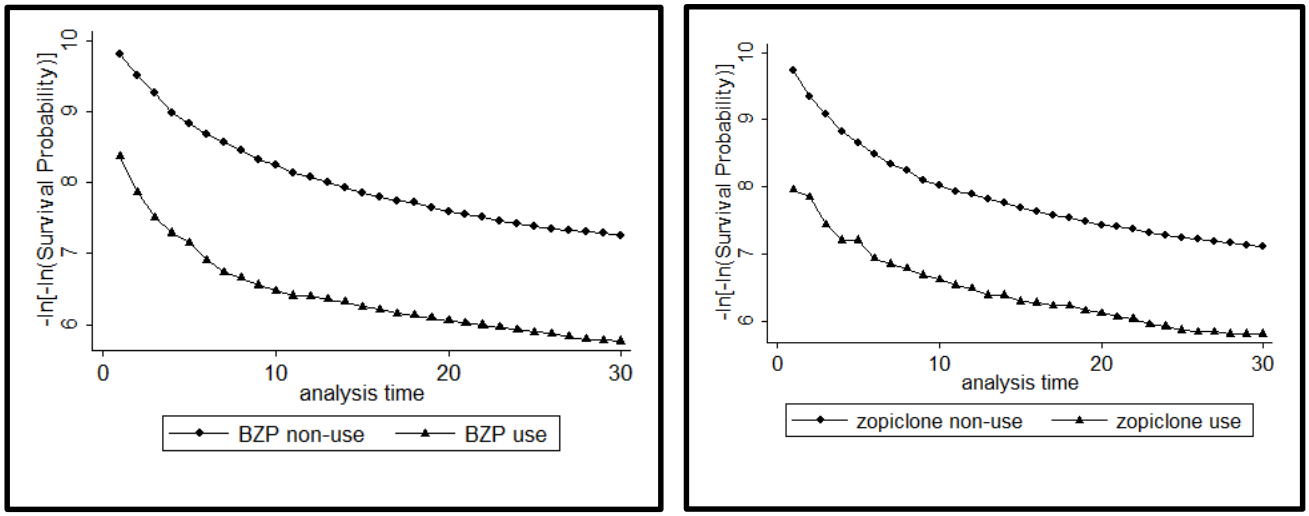


Table 6.4: Global test for proportional Hazards assumption

Drug	P value (Global test)	
	ILI-related pneumonia	**ILI-related death**
Benzodiazepines	0.32	0.04
Zopiclone	0.77	0.22

6.4.3 Benzodiazepines/zopiclone and ILI-related pneumonia

Figure 6.3 presents survival curves, fully adjusted for all covariates as listed above in section 6.3.5 and plotted for the 30-day follow-up period, comparing ILI-related pneumonia risk in benzodiazepine users and non-users (Figure 6.3a); and zopiclone users and non-users (Figure 6.3b). The adjusted survival curves indicate increased pneumonia risk with benzodiazepine use as well as zopiclone use.

Table 6.6 presents hazard ratios for the association between study drugs and occurrence of pneumonia within 30 days following ILI diagnosis. Benzodiazepine use at any time before ILI diagnosis was associated with a 35% increase in the risk of developing ILI-related pneumonia compared to not using benzodiazepines (adj. HR 1.35; 95% CI 1.12-1.63). Increased ILI-related pneumonia was observed among both short term and long term users of benzodiazepines (short term use: adj. HR 1.28; 95% CI 1.05-1.56, long term use: adj. HR 3.14; 95% CI 1.76-5.60). Among individual benzodiazepines, similar statistically significant increases in ILI-related pneumonia were found with diazepam and lorazepam use but not with use of temazepam.

The observed increase in the occurrence of ILI-related pneumonia with zopiclone use lacked statistical significance (adj. HR 1.18; 95%CI 0.89-1.56).

Figure 6.3: Adjusted survival curves for the association between study drugs and ILI-related pneumonia

Fig 6.3a Survival stratified by benzodiazepine use

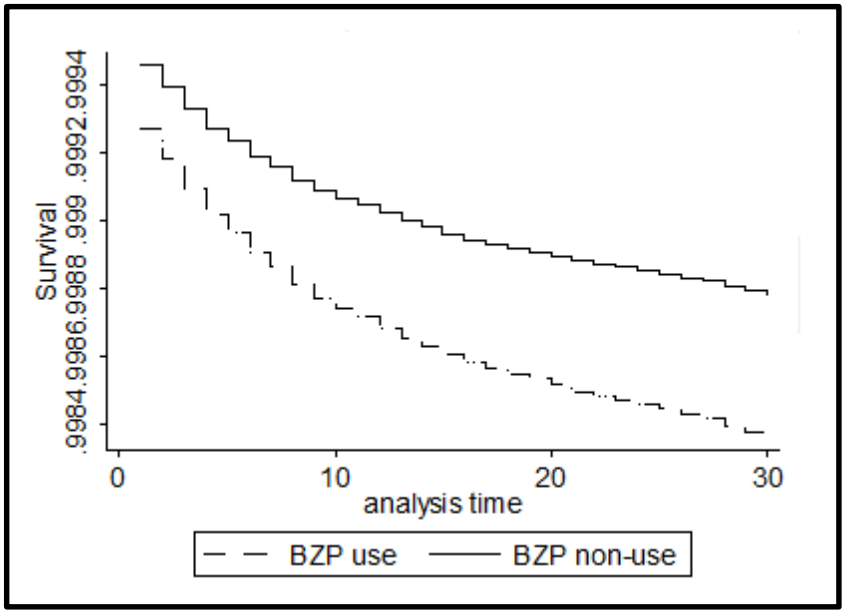


Fig 6.3b Survival stratified by zopiclone use

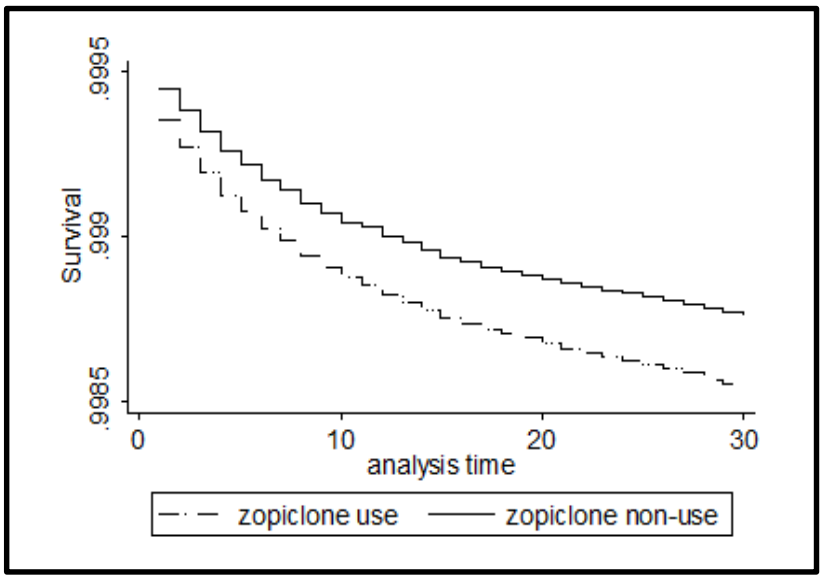


Table 6.5: Association between benzodiazepines/zopiclone use and ILI-related pneumonia in all ages (n= 804,051)

Drug	ILI-related pneumonia Yes (n=1,117)	Unadjusted model HR(95% CI)	Adjusted model HR(95% CI)	
			Model 1	Model 2
Benzodiazepines				
Non-use	981(0.13)	1.00	1.00	1.00
Use	136(0.23)	1.72(1.43-2.05)	**1.33(1.10-1.60)**	**1.35(1.12-1.63)**
Benzodiazepines†				
Current	10(0.70)	5.43(2.91-10.12)	**4.17(2.23-7.79)**	**4.24(2.27-7.95)**
Recent	6(0.30)	2.28(1.02-5.09)	1.79(0.80-4.00)	1.84(0.82-4.11)
Past	120(0.21)	1.60 (1.33-1.94)	**1.24(1.02-1.51)**	**1.26(1.03-1.54)**
Benzodiazepines duration				
Short-term use	124(0.21)	1.61(1.34-1.94)	**1.26(1.04-1.53)**	**1.28(1.05-1.56)**
Long-term use	12(0.68)	5.21(2.95-9.21)	**3.10(1.74-5.51)**	**3.14(1.76-5.60)**
Diazepam				
Non-use	1016(0.13)	1.00	1.00	1.00
Use	101(0.21)	1.61(1.31-1.97)	**1.25(1.01-1.54)**	**1.27(1.03-1.57)**
Diazepam timing†				
Current	4(0.42)	3.17(1.19-8.47)	2.46(0.92-6.57)	2.52(0.94-6.76)
Recent	6(0.39)	2.95(1.32-6.59)	**2.36(1.05-5.27)**	**2.43(1.09-5.44)**
Past	91(0.20)	1.53(1.23-1.89)	1.19(1.05-5.27)	1.21(0.97-1.51)
Temazepam				
Non-use	1065(0.14)	1.00	1.00	1.00
Use	52(0.21)	1.56(1.18-2.06)	1.09(0.82-1.46)	1.11(0.83-1.49)
Temazepam timing†				
Current	5(0.97)	7.26(3.02-17.48)	**5.15(2.13-12.45)**	**5.19(2.15-12.57)**
Recent	1(0.16)	1.15(0.16-8.14)	0.83(0.12-5.89)	0.85(0.12-6.05)
Past	46(4.20)	1.45(1.08-1.94)	1.01(0.75-1.338)	1.03(0.76-1.40)
L**orazepam**				
Non-use	1105(0.14)	1.00	1.00	1.00
Use	12(0.40)	2.95(1.67-5.20)	**2.02(1.14-3.59)**	**2.08(1.17-3.70)**
Lorazepam timing‡†				
Current	2(2.47)	-		**-**
Recent	0(0.00)	-		**-**
Past	10(0.36)	-		**-**
Zopiclone				
Non-use	1063(0.14)	1.00	1.00	1.00
Use	54(0.21)	1.55(1.18-2.04)	1.16(0.87-1.54)	1.18(0.89-1.56)
Zopiclone timing†				
Current	3(0.34)	2.56(0.82-7.94)	1.95(0.63-6.07)	1.97(0.63-6.12)
Recent	2(0.20)	1.49(0.37-5.98)	1.13(0.28-4.52)	1.16(0.29-4.65)
Past	49(0.21)	1.52(1.14-2.02)	1.13(0.84-1.52)	1.15(0.85-1.55)

- † Reference group is the non-user category of the respective drug
- Model 1 adjusted for age, sex, Charlson's comorbidity index score, insomnia and other sleeping disorders, alcohol consumption, current smoking, body mass index and Index of Multiple Deprivation; Model 2: Model 1 covariates, sex, anxiety, stress, depression, psychosis, opioids, steroids, statins, antibiotics, antivirals and FLU vaccination
- Statistically significant results are highlighted in bold
- Drug non-users are the comparison group
- ‡the effect of drug timing was not calculated due to insufficient numbers of respective drug use within the categories.

6.4.4 Benzodiazepines/zopiclone and ILI-related mortality

Figure 6.4 presents the survival curves, adjusted for all covariates as listed in section 6.3.5 and plotted for the 30-day follow-up period, comparing survival in benzodiazepine users and non-users (Figure 6.4a); and zopiclone users and non-users (Figure 6.4b). The adjusted survival curves indicate increased mortality with benzodiazepine use as well as zopiclone use.

Benzodiazepine use at any time prior to ILI diagnosis was associated with a 63% increase in ILI-related death (adj. HR 1.63; 95% CI 1.37-1.93). The observed effect was strengthened with an approximately twenty-fold increase in mortality with current use of benzodiazepines (adj. HR 20.69; 95% CI15.54-27.54). The increase in 30-day mortality was also observed among current users of individual benzodiazepines as well as zopiclone (Table 6.7).

Figure 6.4: Adjusted survival curves for the association between study drugs and ILI-related mortality

Fig 6.4 a) survival stratified by benzodiazepine use

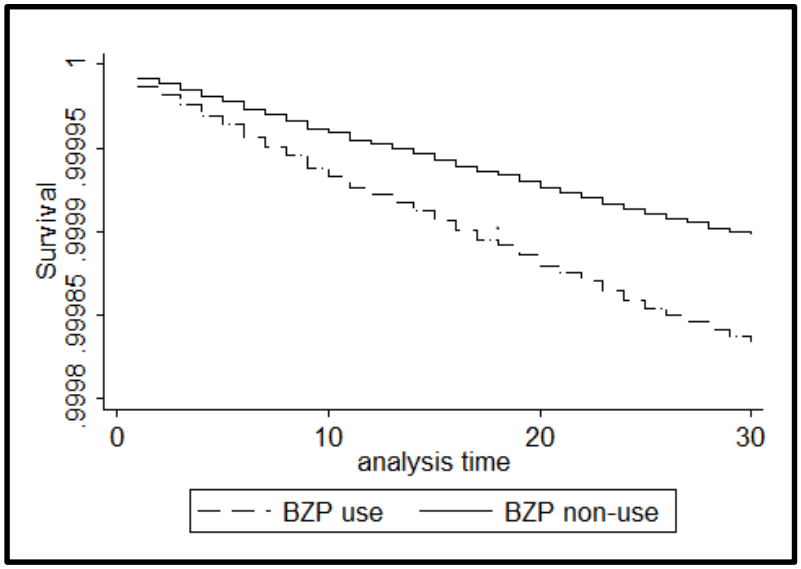


Fig 6.4 b) Survival stratified by zopiclone use

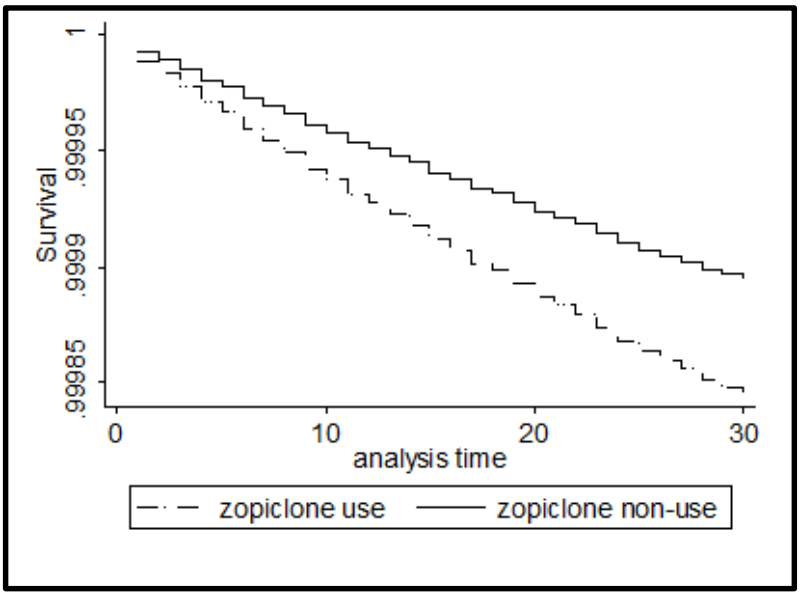


Table 6.6: Association between benzodiazepines/zopiclone use and ILI-related mortality in all ages (n= 804,051)

Drug	ILI-related death Yes (n=707)	Unadjusted model HR(95% CI)	Adjusted model HR(95% CI)	
			Model 1	Model 2
Benzodiazepines				
Non-use	521(0.07)	1.00	1.00	1.00
Use	186(0.31)	4.42(3.74-5.22)	**1.60(1.35-1.89)**	**1.63(1.37-1.93)**
Benzodiazepines				
Current	55(3.84)	57.43(43.50-75.83)	**20.63(15.53-27.42)**	**20.69(15.54-27.54)**
Recent	12(0.60)	8.64(4.87-15.31)	**3.41(1.92-6.06)**	**3.38(1.90-6.01)**
Past	119(0.21)	2.99(2.45-3.65)	1.09(0.89-1.33)	1.11(0.91-1.34)
Benzodiazepines duration				
Short-term use	142(0.24)	3.47(2.89-4.18)	**1.31(1.09-1.58)**	**1.34(1.11-1.61)**
Long-term use	44(2.48)	36.34(26.72-49.44)	**5.57(4.07-7.60)**	**5.58(4.09-7.63)**
Diazepam				
Non-use	600(0.08)	1.00	1.00	1.00
Use	107(0.23)	2.88(2.34-3.54)	1.12(0.91-1.38)	1.15(0.93-1.41)
Diazepam timing				
Current	23(2.41)	31.32(20.65-47.51)	**11.85(7.76-18.08)**	**12.36(8.09-18.90)**
Recent	5(0.33)	4.18(1.73-10.09)	1.91(0.79-4.61)	1.95(0.80-4.71)
Past	79(0.18)	2.24(1.77-2.83)	0.88(0.69-1.11)	0.90(0.71-1.14)
Temazepam				
Non-use	621(0.08)	1.00	1.00	1.00
Use	86(0.35)	4.42(3.53-5.54)	**1.36(1.08-1.72)**	**1.38(1.09-1.75)**
Temazepam timing				
Current	24(4.64)	61.12(40.66-91.90)	**20.10(13.27-30.42)**	**19.56(12.85-29.78)**
Recent	4(0.62)	7.86(2.94-21.01)	2.41(0.90-6.48)	2.30(0.86-6.20)
Past	58(0.25)	3.13(2.39-4.09)	0.97(0.73-1.27)	0.98(0.75-1.30)
Lorazepam				
Non-use	676(0.08)	1.00	1.00	1.00

Use	31(1.05)	12.46(8.69-17.85)	**3.30(2.30-4.75)**	**3.37(2.34-4.85)**
Lorazepam timing				
Current	9(11.11)	149.05(77.21-287.10)	**35.11(18.12-68.05)**	**34.82(17.95-67.55)**
Recent	7(6.03)	74.61(35.43-157.11)	**16.77(7.93-35.45)**	**15.70(7.39-33.35)**
Past	15(0.54)	6.43(3.85-10.72)	**1.72(1.03-2.88)**	**1.76(1.05-2.95)**
Zopiclone				
Non-use	631(0.08)	1.00		1.00
Use	76(0.30)	3.68(2.90-4.67)	**1.46(1.14-1.86)**	**1.48(1.16-1.89)**
Zopiclone timing				
Current	20(2.28)	29.32(18.77-45.76)	**10.81(6.90-16.93)**	**10.86(6.93-17.02)**
Recent	7(0.71)	8.86(4.21-18.67)	**3.61(1.70-7.63)**	**3.61(1.70-7.64)**
Past	49(0.21)	2.56(1.91-3.42)	1.01(0.75-1.36)	1.02(0.76-1.38)

- Drug non-users are the comparison group
- Model 1 adjusted for age, Charlson's comorbidity index score, anxiety, sleeping disorders, opioids, alcohol consumption, current smoking, body mass index, statins, antivirals and Flu vaccination
- Model 2 adjusted for all Model variables plus sex, stress, depression, psychosis, Multiple Deprivation, steroids and antibiotics
- Statistically significant results are highlighted in bold

6.4.5 Sensitivity analyses

Low numbers of patients with ILI-related pneumonia/mortality (outcomes of interest) were observed across the various exposure categories. For instance, only 6 subjects and 12 subjects with recent exposure to benzodiazepines experienced ILI-related pneumonia and ILI-related death respectively (Table 6.6 and 6.7). Simulation studies suggest that there should be a minimum number of around 10 outcome events per variable included in a regression model for statistically significant associations.[155],[156] To fulfil this criteria, current and recent categories of benzodiazepines use were combined, for one of the sensitivity analyses, so that >10 events would be present per category in the regression model. Cox regression analysis results for the recoded benzodiazepine use variable (prescription of benzodiazepines within 90 days prior to the diagnosis of ILI compared to benzodiazepines non-use) show statistically significant increased

risk of ILI-related pneumonia and mortality (ILI-related pneumonia results: adj HR 2.84; 95% CI1.73-4.69; ILI-related mortality results: adj HR 10.78 95% CI 8.30-14.01).

Given benzodiazepines and other GABAergic drugs are commonly used as hypnotics and anxiolytics in adults,[143, 144] a sensitivity analysis was conducted among study subjects aged 18 years and older. Results from this sensitivity analysis for the association of any time benzodiazepines use and ILI-related pneumonia were similar to the original ones (sensitivity analysis results: adj HR 1.34; 95% CI 1.11, 1.62; P=0.003, original results: adj HR 1.35; 95% CI 1.12, 1.63; P=0.002). The sensitivity analysis results for ILI-related mortality were also similar to the original ones (sensitivity analysis results: adj HR 1.81; 95% CI 1.52, 2.15; P<0.001, original results: adj HR 1.98; 95% CI 1.67, 2.36; P<0.001).

For the outcome ILI-related mortality, we conducted a sensitivity analysis to account for severity of illness (a diagnosis of pneumonia within 30 days following an ILI diagnosis was considered as a proxy indicator of ILI severity given most fatal cases of influenza are associated with lower respiratory tract infections, particularly pneumonia).[36] 1,117 patients had pneumonia during the 30-day follow-up period. A subgroup analysis excluding these patients from the main analysis model (anytime benzodiazepine use) yielded comparable results for ILI-related mortality to the original ones

(subgroup analysis results: adj HR 1.68; 95% CI 1.40, 2.01; p<0.001, original results adj HR 1.63; 95% CI 1.37, 1.93; P<0.001).

6.5 Discussion

6.5.1 Summary of main findings

Results from this study indicate that benzodiazepine use is associated with increased occurrence of both ILI-related pneumonia and mortality. The increased risk is present with long term as well as short term use of benzodiazepines. This effect is also present across the individual benzodiazepines assessed, diazepam, temazepam and lorazepam. Zopiclone, the non-benzodiazepine hypnotic with the same mechanism of action as benzodiazepines, was also found to be associated with increased ILI-related mortality but not pneumonia.

6.5.2 Strengths and limitations

Given almost all ILI cases are managed in the community, using general practice data allowed access to a large sample of ILI patients (n=504,051) as well as to a wide range of data pertaining to patient prescription and medical history. As these were cohort data, temporal biases relating to timing of exposure were minimised. This study also adjusted for a wide range of potential confounders. Confounding by comorbidity was controlled for using a robust measure, the Charlson comorbidity index, designed specifically to predict mortality given a set of comorbid diseases.[157] This comorbidity measure has also been

validated for use in morbidity studies.[158] In addition, other illnesses identified in literature as confounders of the relationship between benzodiazepines and disease outcomes were controlled for. These include; insomnia and sleep disorders, depression, psychosis, anxiety and stress.[104, 126] Furthermore, other drugs that could be concurrently prescribed with benzodiazepines (e.g. opioids and corticosteroids) and life style factors (e.g. smoking and alcohol consumption) were controlled for.

A possible limitation of this study may be an overestimation of exposure to study drugs given prescription data, which does not guarantee drug compliance, was used to determine exposure status. Whilst this could lead to misclassification bias, the effects are likely to be non-differential and would therefore bias results towards the null. Nevertheless, the observed associations still remained when chronic benzodiazepine use as indicated by repeat prescriptions was assessed (Table 6.6 and 6.7). Another limitation is the possibility of unmeasured and residual confounding inherent to observation studies especially those that utilise routine databases.[159] In addition, it is possible that the outcomes of interest were more likely to be recorded in people who sought medical attention because they were more anxious about pre-existing comorbidities or their health in general; these individuals may be more likely to be prescribed medications for anxiety that include benzodiazepines than those who did not seek medical attention because they were generally well. While an attempt

has been made to account for comorbidity burden and conditions like anxiety and stress separately, there may still be an inadvertent selection bias that cannot be fully corrected for.

6.5.3 Comparison with existing literature

Our finding that use of benzodiazepines at any point in time before influenza like illness is associated with a 35% increased occurrence of ILI-related pneumonia is slightly lower than the 54% increased pneumonia risk reported by Obiora et al[126] who assessed the effects of these drugs on pneumonia risk in the UK general population. One possible reason for the smaller point estimate found in our study may be attributable to the presence of very few current (10/136) and recent users (6/136) among the study drug users with the outcome of interest. As shown in table 6.6, the majority of exposed patients with ILI-pneumonia were past benzodiazepine users (120/136). Although past benzodiazepine use was shown to have a statistically significant association with increased occurrence of pneumonia in the Obiora et al study, the effect established was weaker than for the current and recent drug use.[126]

The same issue of having mainly past study drug users in the 'any time use' category (49/54) may also explain the lack of statistical significance for the observed effect of zopiclone on ILI-related pneumonia.

On the other hand, mortality estimates for use of study drugs at any point in time prior to ILI diagnosis observed in the present study are comparable to findings from some previous studies.[103, 110, 153, 160] However, the present estimates of current benzodiazepine/zopiclone use are higher than reported in the available literature (Chapter 2; Table 2.3). The higher mortality point estimates also persisted in the sensitivity analysis that combined current and recent benzodiazepine exposure into a single category to boost numbers of exposed patients with the outcome of interest (ILI-related mortality sensitivity analysis results: adj HR 10.78 95% CI 8.30-14.01; original results: adj HR 20.69; 95% CI 15.54-27.54). A plausible explanation for the differences in mortality estimates observed in the present study compared to previous studies could be the different study populations utilised. Unlike previous studies undertaken in the general population,[103-105] the present study assessed the effects of benzodiazepines in a cohort of patients with underlying physician diagnosed influenza like illness. Benzodiazepine use in influenza has been suggested to influence susceptibility to the underlying infection as well as bacteria superinfection in animal models of infection.[58, 74] As this study is the first of its kind, further studies are required to supplement these findings.

6.5.4 Summary

This study shows a statistically significant association between benzodiazepines use and increased occurrence of ILI-related

pneumonia and mortality. Given the low numbers for drug use among patients with ILI-related pneumonia and ILI-related mortality (outcomes of interest), particularly current drug users, additional studies with sufficient numbers of exposed patients with the outcomes of interest are required to investigate this research question further.

Chapter 7 : Discussion of thesis findings, conclusion and recommendations

7.1 Introduction to this chapter

This thesis set out to build on the previous evidence from pre-clinical and clinical studies, which suggested that benzodiazepines increase vulnerability to infection (both primary and secondary infection) and mortality, by studying UK primary care patients with asthma or influenza-like illness (ILI). An additional post-hoc analysis of the non-asthma primary care patient population was undertaken in response to reviewers' comments on the asthma study manuscript submitted to the Lancet Respiratory Medicine journal. This allowed a greater insight into the possible mechanism underlying the observed association between benzodiazepines and mortality. To summarise, the thesis objectives were as follows:

6) To investigate the association between benzodiazepines and exacerbation of asthma in patients with asthma
7) To investigate the association between benzodiazepines and mortality subsequent to asthma exacerbation in patients with asthma
8) To investigate the association between benzodiazepines and mortality in patients without asthma
9) To investigate the association between benzodiazepines and ILI-related pneumonia in patients with ILI

10) To investigate the association between benzodiazepines and ILI-related mortality in patients with ILI

Presented below in sections 7.2 to 7.4 are a summary of the main findings and an overall discussion of these findings, conclusions based on the work undertaken in this thesis and recommendations for practice and future research.

7.2 Summary of main thesis findings

Figure 7.1 below presents a summary of the main thesis findings. In people with asthma, current use of benzodiazepines was associated with a 49% statistically significant increase in the occurrence of asthma exacerbation compared to benzodiazepines non-use. Analyses of zopiclone, a non-benzodiazepine drug with a similar mechanism of action as benzodiazepines which were undertaken to determine the effect of GABAergic drugs as a group, showed a 59% increased occurrence of asthma exacerbation with current use which was also statistically significant. Similarly, an assessment of the individual benzodiazepines revealed an increased likelihood of occurrence of asthma exacerbation with diazepam and temazepam but not lorazepam.

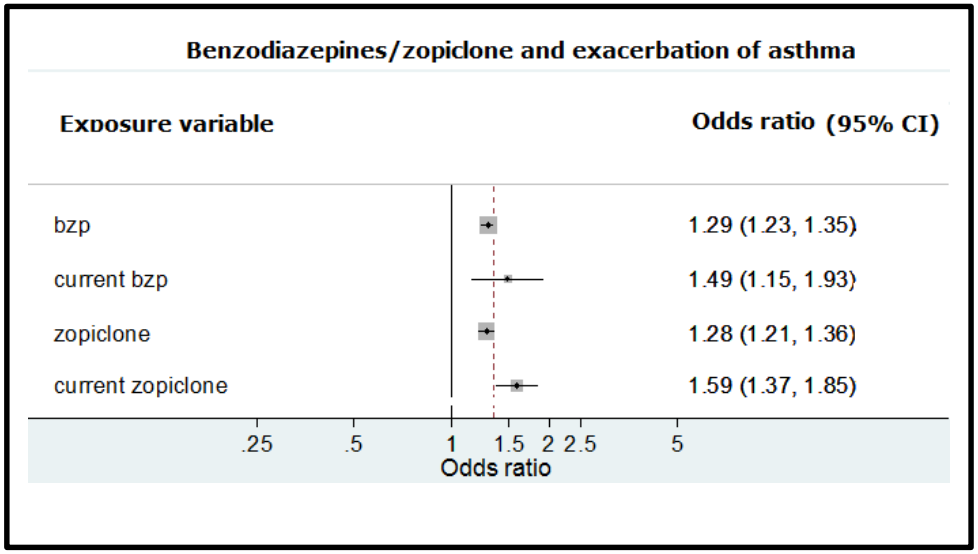
A positive association between benzodiazepine use and increased pneumonia risk was also identified among patients with physician diagnosed ILI. ILI patients with a current exposure to benzodiazepines were more than four times likely to develop

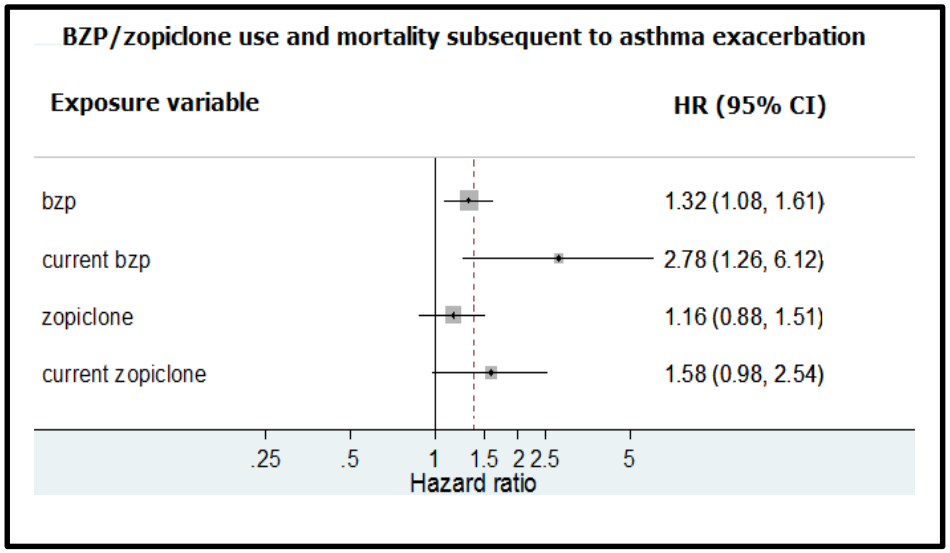
pneumonia during 30 days of follow-up compared to non-users of benzodiazepine. The increased ILI-related pneumonia risk was similarly observed with current use of the three individual benzodiazepines; diazepam, lorazepam and temazepam. The ILI-related pneumonia risk associated with zopiclone use did not achieve statistical significance.

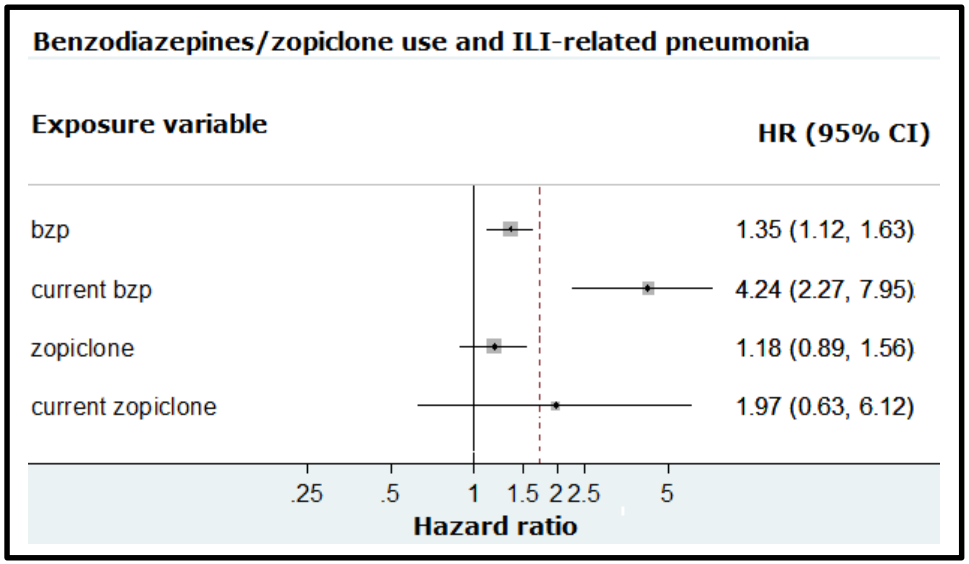
A relative increase in mortality risk was found with benzodiazepine use at any point in the patient's history compared to non-use in the different study populations assessed in this research namely asthma patients, patients without asthma and ILI patients. A 33% increase in mortality subsequent to asthma exacerbation, 63% increased mortality in non-asthma and 63% increase in ILI-related mortality in patients with ILI were observed among benzodiazepines users compared to non-users of these drugs. An assessment of current benzodiazepine use revealed a twenty fold increase in the occurrence of ILI-related mortality. An increase in mortality with all the three individual benzodiazepines assessed (diazepam, temazepam and lorazepam) as well as zopiclone was also observed in the non-asthma population. Temazepam, lorazepam and zopiclone but not diazepam showed a statistically significant association with increased ILI-related mortality whereas only diazepam achieved statistical significance with increased mortality subsequent to asthma exacerbation.

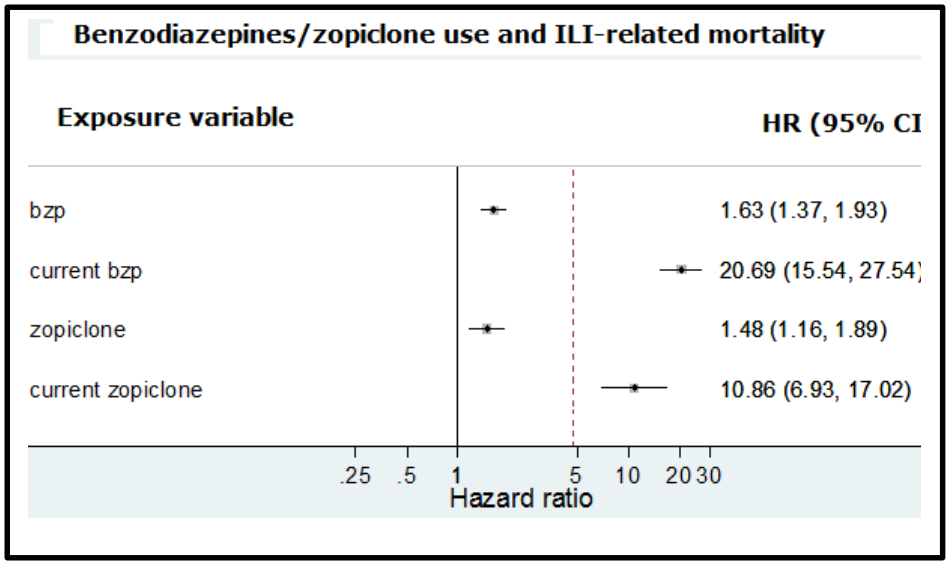
Both short term and chronic exposure to benzodiazepines was associated with increased risk of asthma exacerbation, ILI-related pneumonia as well as mortality in the respective patient population groups assessed.

Figure 7.1: Summary of the overall thesis findings









7.3 Overall discussion of thesis findings

The research studies presented in this thesis supplement findings from other well conducted studies to date.[104, 105, 126] The findings from the current study of the association between benzodiazepines and ILI-related pneumonia are in line with results from Obiora et al[126] study which also observed a statistically significant association between increased pneumonia risk and benzodiazepine use though of a lower effect size (effect of current benzodiazepine use and pneumonia risk: Obiora et al. adj. OR 1.89; current study adj. HR 4.24). Conversely, Dublin et al[98] did not report an increased risk of pneumonia with exposure to benzodiazepines. Their study however was undertaken in patients over 65 years old, which differs from the study population used in Obiora et al and the current study of patients of all age-groups. It is well known that old age is related to increased comorbidity. In Obiora et al and the current study, increased comorbidity was found to be associated with a smaller effect of benzodiazepines than in patients with lower comorbidity burden, an observation that may be explained by the impact of cumulative disease processes in offsetting the proportion of the risk attributable to the drug. Thus, the non-significant association established in the study by Dublin et al. may be explained, at least in part, by the comorbidity associated with old age. In addition, Dublin et al reported

wide confidence intervals that may indicate low numbers of patients exposed to benzodiazepines in their study.

The positive association between benzodiazepine use and increased infection risk (ILI-related pneumonia) observed in the current study is also supported by findings from animal and clinical studies.[58, 161] For instance, treatment with diazepam increased susceptibility to Streptococcus pneumonia superinfection in influenza infected C57BL/6 mice.[58] Similarly, in critically ill patients increased risk of secondary infection was reported in sedation with benzodiazepine midazolam relative to dexmedetomidine (19.7 vs. 10.2%; p = 0.02).[161] However, in such clinical studies of benzodiazepines sedation, high doses of benzodiazepines were used, limiting generalisability of such findings to the current research and other epidemiological studies which assessed benzodiazepine use in the community.[73, 97, 98]

Increased infection risk has also been found with use of the non-benzodiazepine GABAergic hypnotics, including zopiclone, in the meta-analysis of data from randomised controlled trials[91] and by Obiora et al.[73] Although the current study equally observed an increased occurrence of ILI-related pneumonia with Zopiclone use, this association failed to achieve statistical significance. A possible explanation may be the lack of statistical power as low numbers of patients were exposed to zopiclone in this study.

With regards to benzodiazepines/zopiclone use and mortality risk, the current results of increased mortality with benzodiazepine/zopiclone use in over 180,000 subjects in the general population without physician diagnosed asthma are in line with recent findings from equally large prospective general population studies.[104, 105] In these studies, the effect size of benzodiazepines on mortality ranged from HR 1.26-4.43 (Chapter 2: Table 2.3).

On the other hand, studies focusing on specific population groups showed conflicting results though the majority reported increased mortality risk with benzodiazepine use that was not statistically significant. Population groups so far studied include patients with dementia[162], pneumonia,[73, 163] renal disease,[112] Parkinson disease[109] and chronic obstructive pulmonary disease (COPD).[153] Overall, the use of benzodiazepines was statistically significantly associated with increased mortality risk (Chapter 2: Table 2.3); these findings are in keeping with the current studies of benzodiazepine effects in patients with asthma or influenza-like illness. In addition, exposure to benzodiazepines was found to be associated with increased exacerbation of asthma in patients with asthma. Although it is not possible to make a direct comparison of these results with earlier findings as this is the first study of its kind, a similar observation was made for patients with COPD where benzodiazepines increased outpatient exacerbations and visits to emergency room.[132]

Based on recent suggestions that the detrimental effect of benzodiazepine on disease outcomes is dependent on GABAA receptors signalling on monocytes/macrophages which results in impairment of cytokine release, phagocytosis and bacterial killing by phagocytes,[58] a similar effect across the individual benzodiazepines and zopiclone on outcomes of interest was hypothesised in the current studies given all benzodiazepines and Z-drugs have similar actions on GABA receptors.[41] However, not all the assessed individual benzodiazepines and zopiclone showed a consistent effect across all outcomes of interest, an observation also found in a similar previous study which assessed the impact of benzodiazepine on community acquired pneumonia and subsequent mortality.[126] In both studies the highest risks were observed in relation to use of benzodiazepines, particularly diazepam. The diazepam finding may be attributable to its prolonged half-life and active metabolites.[46] An alternative explanation may be that diazepam is commonly used and so enough study subjects were more likely to be exposed to diazepam than other benzodiazepines. Thus, the non-significant association observed for other individual benzodiazepines as well as zopiclone with some outcomes of interest could be due to relatively low usage in the population studied and that probably there is a drug class effect that these studies failed to detect. Additional studies are however still needed to further investigate this assumption of a class drug effect.

Given the strengths of this current research including adjustment for a wide range of confounders, the persistently observed adverse outcomes associated with benzodiazepine use suggest, at the minimum, a benzodiazepine adverse effect signal. However, the current research does not claim a causal relationship partly because of the inherent limitation of observational studies such as confounding by indication, unmeasured and residual confounding. Interestingly though, Vanderbroucke's[164] eloquent arguments published in The Lancet in 2004 draw attention to the strengths of pharmacoepidemiology in detecting drug adverse effects as he notes that "*The average randomised drug trial is too small, and does not have sufficient follow-up to detect adverse effects that are fewer than about one per 200 per year, or that take longer than 1 year to develop"*, and therefore, he goes on to state that either case-control studies or large-scale observational follow-up studies are needed. The second interesting point he makes is that, "*when unintended adverse drug effects are studied, the possibility of 'confounding by indication' is low, "When the prescriber is effectively blind to the prognosis (i.e., the risk) of the patient developing the adverse effect, this achieves the same aim of breaking the link between prescribing and prognosis"."*[164]

In this regard, it is worth reviewing the observed association that benzodiazepines increase infection/mortality risk using the Bradford Hill criteria for causation[165] as a framework for further scrutiny of this

association. The Bradford Hill Criteria for causation comprise nine aspects of an association that should be considered when deciding whether the likely interpretation of any association is causation. These are: strength of an association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experimental evidence and analogy. A brief application of each of these aspects to the observed association between benzodiazepines and disease outcomes in the current research is presented below:

1) Strength of association

It is generally accepted that the stronger the association the more likely it is a reflection of causal relationship.[166] It is however also recognised that a true casual factor may be related to a small risk increase in cases where such a factor is one of the many factors operating to cause the outcome.[167] The effect size of three or less in epidemiological studies is considered moderate/small,[168, 169] and considering that the estimated effect size for benzodiazepine use was mostly less than two in the current research (except for current benzodiazepine use and the occurrence of ILI-related pneumonia and mortality), it is therefore concluded that the strength of observed association between benzodiazepines and outcomes of interest is generally weak.

2) Consistency of association

This aspect of Bradford Hill criteria for causation is concerned with reproducibility of findings by different researchers under different conditions though a lack of consistency does not rule out a casual association because some effects may be produced only in certain circumstances.[165] The current research findings for the effect of benzodiazepines and mortality are in keeping with previous mortality studies in the general population[104, 105] but not for the elderly population.[106-108] The contrast between results from the elderly population and general population suggests heterogeneity across the different study populations. This however raises an intriguing question as to whether the effect of benzodiazepines can only be detected under certain circumstances for instance in the presence of lower comorbidity as observed in subgroup analyses in the current research. However, some previous studies in other population groups also did not establish a link between benzodiazepine use and increased risk of pneumonia.[99, 170]

3. Specificity of association

This argues that an exposure which influences a single outcome is good evidence for causality but also acknowledges that this may not be the case in certain circumstances.[167] For example, this concept of specificity may be misleading in some pharmacovigilance studies as most drugs have multiple side effects. The latter case is true for the

current research findings as benzodiazepines are capable of producing multiple side effects.

4. Temporality of association

In this concept of causality, the exposure of interest must precede the outcome under study.[166] This criterion was met in all the three studies presented in this thesis (Chapter 4-6). Previous research has also demonstrated the presence of benzodiazepine use before reports of its detrimental effects on disease outcomes.[73]

5. Biological gradient

A biological gradient in epidemiological study is synonymous with a dose-response relationship whereby the outcome increases with increasing dose of exposure.[166] Although this study did not analyse benzodiazepines dose data, duration of exposure was considered and higher point estimates were found with long term use of benzodiazepines than short-term use for the ILI-related pneumonia and mortality risk. Two recent studies which examined dose response effect have shown increased risk of mortality with increasing dose of benzodiazepines.[104, 105]

6. Plausibility

In this aspect of causal relationship, the observed association ought to have a plausible explanation, in most cases a biologically plausible mechanism of action.[166, 167] Recent data suggest that benzodiazepines effect on disease outcomes is dependent on GABAA receptors

signalling on monocytes/macrophages impairing cytokine release, phagocytosis, and bacterial killing by macrophages, hence the increase in vulnerability to infection.[58] In addition, benzodiazepines actions on GABAA receptors are thought to be involved in the pathogenesis of asthma and immunity in the lungs.[58, 135]

7. Coherence

The concept of coherence, as an aspect of a causal association, requires that the conclusion made about the observed association between exposure and the disease/disease outcome should not have profound contradictions of present 'generally known facts of natural history and biology of disease' (disease outcomes).[165] It should be noted that the current research area of benzodiazepines and infection/mortality risk is relatively new and so the knowledge against which the observed associations are evaluated is still developing. The current research findings though are in agreement with the expected direction of association given the currently suggested mechanism of action i.e. increased infection/mortality risk with benzodiazepine use due to impairment of immune response to infection via its action on GABAA receptors located on immune cells.[58]

8. Experimental evidence

As noted earlier on, there is still need for a large RCT to investigate the observed associations between benzodiazepines and disease

outcomes specifically asthma exacerbation and pneumonia risk, ethical considerations permitting.

9. Analogy

Based on the available evidence from experimental animal studies that benzodiazepines actions on GABAA receptors depress immunity,[58, 83] the current research findings that benzodiazepines are associated with increased infection/mortality risk are analogous to results from studies of other immune-depressive drugs such as corticosteroids.[171] [172, 173]

7.4 Conclusion, recommendations for practice and future research

The association between benzodiazepines and increased asthma exacerbation, ILI-related pneumonia and mortality risk observed in this research signal an adverse benzodiazepine effect. The temporality and biological plausibility in concert with laboratory evidence from elsewhere as well as the analogous evidence supporting increased risk of infection/inflammation with benzodiazepine use possibly mediated via actions on GABAA receptors,[58] add weight to this signal.

It is therefore important that clinicians exercise a precautionary approach to prescribing benzodiazepines/zopiclone during the interim before conclusive evidence is yielded by further research.

As the decision to discontinue a drug must be informed by the evidence of its potential harms and benefits, the findings of this research suggest that it may be worth avoiding these drugs in end-of life care as these drugs have been shown to increase short-term mortality.

Assuming a causal relationship between benzodiazepines and asthma exacerbation and subsequent mortality, the unadjusted NNH results suggest one additional asthma exacerbation within a year of benzodiazepine use for every 22 asthma patients and one additional death for every 17 patients in a median follow-up of two years. Secondly, because influenza-like illness and asthma are common diagnoses and since benzodiazepines are also commonly prescribed in the general practice, even a small increase in the risk of pneumonia or asthma exacerbation attributed to benzodiazepines could have considerable impact on the burden of these diseases. Clinicians therefore should carefully consider their decision to prescribe benzodiazepines or zopiclone especially in patients who may already be at an increased risk of asthma exacerbation or pneumonia.

Future research should be able to address some of the limitations encountered in the current research. Most importantly, prescription data was used to define benzodiazepine use in the current research and indeed in the majority of previous studies (Chapter 2: Table 2.2 and 2.3). This approach does not guarantee the actual use of the drug by the patient. A more robust approach therefore would be necessary

in future studies for instance one that involves linkage of pharmacy databases with diagnostic data to determine prescription fills and refills and to also incorporate follow-up of patients for example with home or clinic visits to determine actual drug use.

Furthermore, this study did not determine mean daily dose for benzodiazepines/zopiclone. Although a dose response relation relationship between hypnotics and mortality has been found in recent studies,[104, 105] this hasn't yet been done for other outcomes like pneumonia and exacerbation of asthma. Future studies would therefore shed light on the effect of different doses of benzodiazepines on infection risk. Additional data is also needed to supplement the evidence on the dose response relationship between benzodiazepines and mortality as it has been suggested that even low usage of benzodiazepines <18 pills per year could be associated with increased mortality.[105] A similar conclusion could be drawn from the findings in this research in which even a one-off prescription was associated with increased mortality in patients without asthma.

Another area of interest in need of further exploration is the effect of individual benzodiazepines as noted earlier on in the overall discussion section above. Future studies to draw a conclusion on whether the observed benzodiazepines effects reflect a class effect or not would need sufficient numbers of subjects per individual benzodiazepines. This also applies to the effect of non-benzodiazepine

drugs, with the same mechanism as benzodiazepines, represented in this study by zopiclone.

This research largely utilised Read codes to define asthma and ILI patients as well as outcomes of interest (asthma exacerbation and pneumonia); a validation study of such diagnoses used in general practice would serve as a useful reference for future studies on pneumonia and outcomes of asthma.

Finally, a change in prescribing policies generally requires clinical trials to determine whether observations made in epidemiological studies can be demonstrated in the relevant experimental studies. Ideally randomised controlled drug trials are preferred to investigate these associations within the appropriate realms of research ethics. Additional evidence may also be obtained by undertaking other large prospective cohort studies employing standardised data collection protocols and/or self-controlled case series that would account for fixed confounding via intra-person comparison.[174]

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Appendices

Appendix 1.0: Literature review search strategy in Medline database

Search number	Search terms	Number of articles	Search Type
Exposure terms			
1	benzodiazepines.mp. or exp Benzodiazepines/	29113	Advanced
2	exp Diazepam/ or diazepam.mp.	7429	Advanced
3	lorazepam.mp. or exp Lorazepam/	1986	Advanced
4	midazolam.mp. or exp Midazolam/	7486	Advanced
5	nitrazepam.mp. or exp Nitrazepam/	223	Advanced
6	alprazolam.mp. or exp Alprazolam/	1075	Advanced
7	chlordiazepoxide.mp. or exp Chlordiazepoxide/	677	Advanced
8	z-drugs.mp.	59	Advanced
9	zopiclone.mp.	541	Advanced
10	zolpidem.mp.	1474	Advanced
11	zaleplon.mp.	289	Advanced
12	hypnotics.mp. or exp "Hypnotics and Sedatives"/	36177	Advanced
13	exp Anti-Anxiety Agents/ or anxiolytic*.mp.	25833	Advanced
14	exp Tranquilizing Agents/ or tranquilisers.mp.	76719	Advanced
Outcome terms			
15	Asthma*.mp. or exp Asthma/	79338	Advanced
16	exp Influenza A virus/ or influenza*.mp. or exp Influenza B virus/ or exp Influenza, Human/	57524	Advanced
17	influenza-like-illness.mp.	1559	Advanced

18	ILI.mp.	1279	Advanced
19	exp Respiratory Tract Infections/ or influenza like illness.mp.	131506	Advanced
20	Pneumonia*.mp. or exp Pneumonia/	88608	Advanced
21	exp Community-Acquired Infections/ or community acquired pneumonia.mp.	11359	Advanced
22	mortality.mp. or exp Mortality/	481594	Advanced
23	exp Death/ or death.mp.	403626	Advanced
24	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14	116048	Advanced
25	16 or 17 or 18 or 19	160751	Advanced
26	20 or 21	92823	Advanced
27	22 or 23	786105	Advanced
28	24 and 15	213	Advanced
29	24 and 25	382	Advanced
30	24 and 26	391	Advanced
31	24 and 27	5586	Advanced

Appendix 2.0: Benzodiazepines and pneumonia risk: a systematic review and meta-analysis of literature

Introduction

Despite the existence of effective strategies for prevention and management of pneumonia, this disease continues to inflict substantial morbidity and mortality worldwide.[175] A growing body of evidence from animal and human studies suggests that benzodiazepine use might adversely influence the risk of developing

infections and more specifically, pneumonia.[58, 74, 83] Therefore a systematic review and meta-analysis of literature was conducted to summarise the impact of benzodiazepine use on pneumonia incidence.

Methods

A broad search was undertaken in Medline, MBASE, PsycINFO (Ovid) and Web of science databases from inception to October 2012 and updated in June 2015.

The following combinations of search terms were used in the Medline database, replicated in MBASE, and modified to suit the search criteria in other databases mentioned above: (benzodiazepines.mp. or exp Benzodiazepines OR exp Diazepam/ or diazepam.mp OR lorazepam.mp. or exp Lorazepam OR midazolam.mp. or exp Midazolam OR nitrazepam.mp or exp Nitrazepam OR alprazolam.mp. or exp Alprazolam OR chlordiazepoxide.mp or exp Chlordiazepoxide OR z-drugs.mp. OR zopiclone.mp OR zolpidem.mp OR zaleplon.mp OR hypnotics.mp.or exp "Hypnotics and Sedatives" OR exp Anti-Anxiety Agents/ or anxiolytic*.mp. OR exp Tranquilizing Agents/ or tranquilisers.mp.) AND (Pneumonia*.mp. or exp Pneumonia OR exp Community-Acquired Infections/ or community acquired pneumonia.mp).

Bibliographies of relevant articles were searched for additional studies. No language restriction was applied and all study designs

were considered. Data were extracted using a piloted form developed by research team members at the University Of Nottingham.

Methodological quality of included studies was evaluated using the Newcastle-Ottawa assessment Scale (NOS).[102] The generic inverse variance method was used to analyse the data using a random effects meta-analysis model to allow for anticipated heterogeneity between the studies, the latter being assessed using the I^2 statistic. All analyses were conducted in Stata 12.0 (Statacorp Inc.).

Results

The search yielded a total of 6159 articles, of which 32 were retained following a review of titles and abstracts. Only 7 observational studies were included in the meta-analysis after a full-text review (Flow chart 2.0.1). No randomised trials were available. Reasons for exclusion from the meta-analysis included assessment of a class-wide effects (e.g. sedatives) from which the effect of benzodiazepines could not be singled out, and animal studies. Table 2.0.1 summarises the selected studies.

Three studies examined pneumonia incidence in older adults (≥ 65 years)[94, 95, 98] while the rest assessed different age groups for instance, the two studies from Almirall et al. were conducted in patients aged 14 years and older.[97, 176] The NOS score for the studies included in the meta-analysis ranged from 5 to 9 (maximum), with

three studies rated as having high methodological quality (NOS ≥ 8).[94, 95, 98]

In the subsequent meta-analysis, benzodiazepine use was associated with a 5% increase in the point estimate of pneumonia risk but this lacked statistical significance (pooled OR=1.05, 95% CI 0.83, 1.32; I^2=87.0%). A restricted analysis of only those studies with high methodological quality revealed a 19% point estimate increase in pneumonia risk, but this also failed to reach statistical significance (OR 1.19; 95% CI 0.86, 1.66; I^2=91.5% [Figure 2.0.2]).

Interpretation of findings

These results do not confirm an increased risk of pneumonia associated with benzodiazepine use. However this does not prove there is no risk as considerable statistical heterogeneity between studies was noted, which could be attributed to differences in study design, populations studied, definitions of benzodiazepine use, and the range of confounders considered. The slight increase in point estimate when only higher quality studies are considered implies that benzodiazepines cannot confidently be given the 'all clear" in clinical practice and that high quality prospective cohort studies are required to adequately resolve their safety in the context of pneumonia risk.

Conclusion

The effect of benzodiazepine on pneumonia risk is unproven and remains inconclusive, even after meta-analysis of available literature.

Figure 2.0.1: Search results for BZP use and pneumonia risk

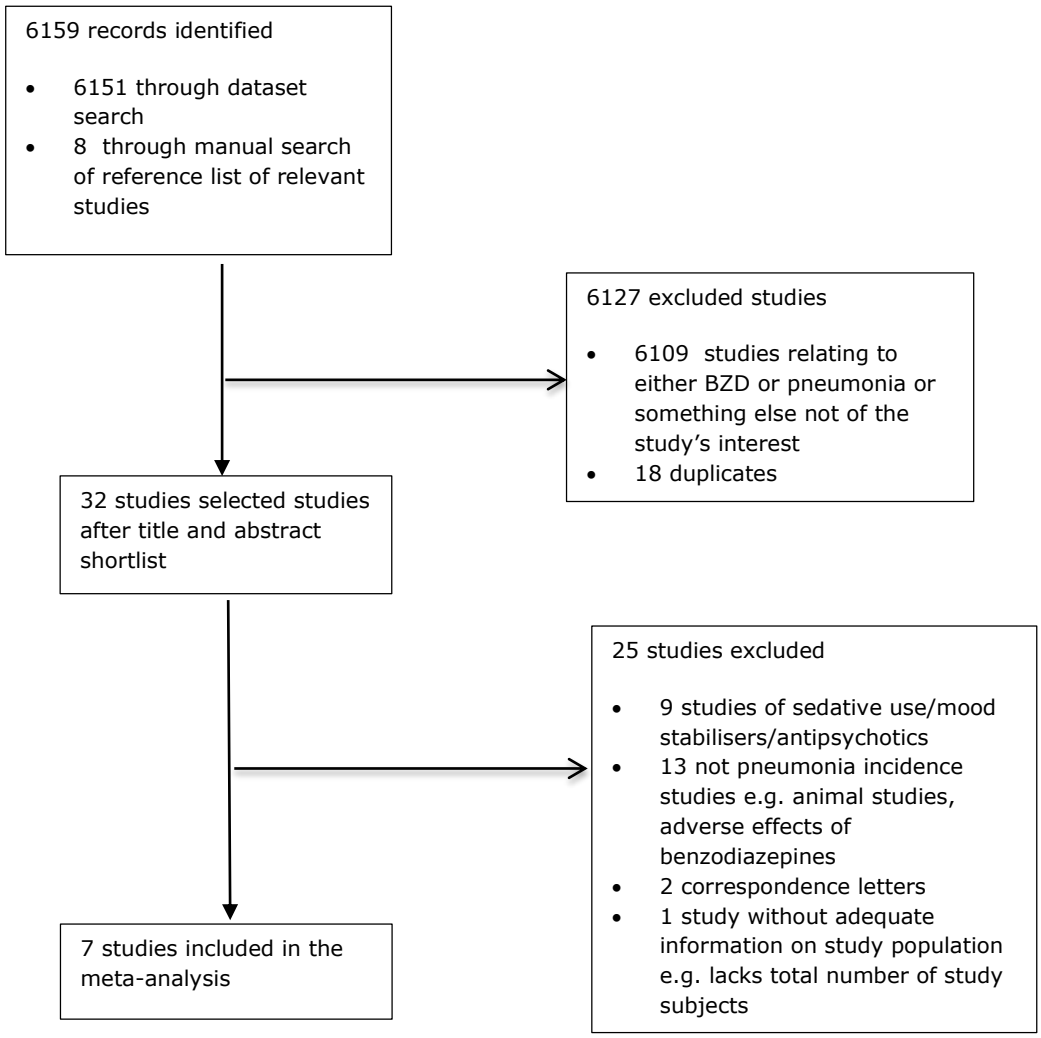


Table 2.0.1: Characteristics of studies[c] of the association between benzodiazepines and community acquired pneumonia

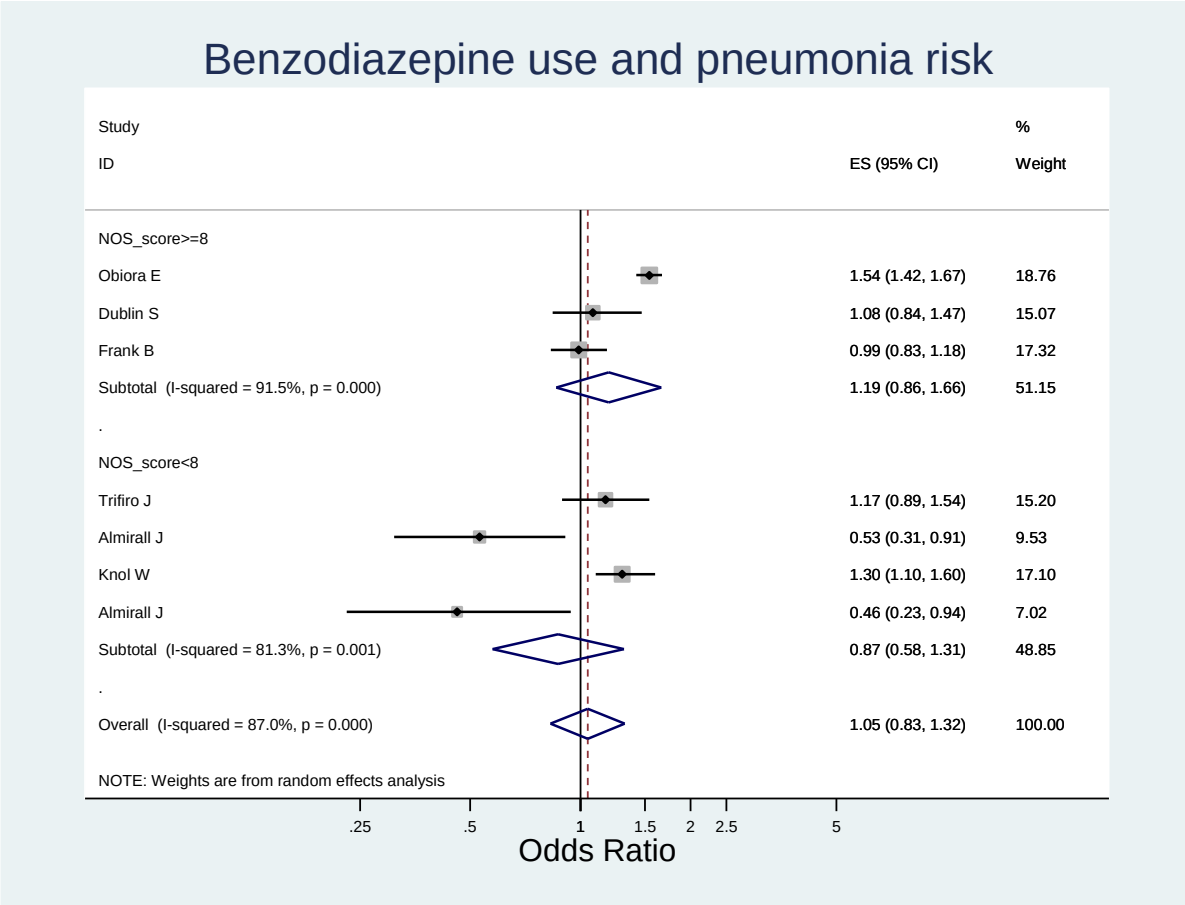
Author/ year	Location/ study period	Primary focus of the study	Definition of benzodiazepine use	Adjustment of confounders	Number of events*/ patients	Odds Ratio (95% CI)	Quality assessment score†
Frank B et al (2014)	UK 1998 to 2008	BZP and stroke outcomes (modified Rankin scale score and pneumonia incidence)	Not randomised benzodiazepine treatment	National Institutes of Health Stroke Score, alteplase in patients with ischaemic stroke and age	779/5846	0·99 (0·83–1·18)	8
×Iqbal U et al (2013)	Taiwan Not specified	BZP and pneumonia incidence	Prescription benzodiazepines in a number of specified periods e.g. 30, 60 and 90	None	56019/not specified	OR 0.46‡	5
Obiora et al 2012	UK 2001-2002	Benzodiazepine and pneumonia incidence	Prescription data (e.g. current use was defined as the most recent prescription within 30 days before the pneumonia index date)	Age, sex, Ischemic heart disease, pulmonary disease, previous pneumonia episodes, Charlson's comorbidity index, alcohol consumption, depression, Psychosis, current smoking and socioeconomic status	4964/34661	1.54(1.42-1.67)	8
Dublin et al (2011)	USA 2000-2003	Effect of opioids or Benzodiazepines on risk of acquiring CAP	Prescription refill data (e.g. Current use was defined as having filled one or more Prescriptions between 5 and 60 days before the index date	Age, sex, asthma, COPD, COPD hospitalisation, use of oxygen, long-term corticosteroid use for lung disease, use of opioids, whether FEV1 was measured, CHF, CHF hospitalisation, whether EF was measured, stroke, number of outpatient visits in 1-year baseline period, need for assistance with ambulation or bathing, dementia and use of inhaled bronchodilators	1039/3061	1.08(0.84-1.47)	9

Author/ year	Location/ study period	Primary focus of the study	Definition of benzodiazepine use	Adjustment of confounders	Number of events*/ patients	Odds Ratio (95% CI)	Quality assessment score†
Trifiro et al (2010)	The Netherlands 1996-2006	Association of CAP with antipsychotic drug use	Previous and concomitant use (within 3 months before date of first specific pneumonia symptom) of benzodiazepines with antipsychotic drugs	Age, sex, date of pneumonia onset and use of antipsychotic drugs	258/1947	1.17(0.89-1.54)	5
Almirall et al (2008)/ 2013 (re-analysis)	Spain 1999-2000	Risk factors for CAP with special emphasis to modifiable risk factors and those applicable to the general population	Regular benzodiazepine treatment during the last year	age, sex, primary centre, asthma, chronic bronchitis, upper airway infection in the last month, hospitalisation in the last 5 years, temperature change, educational level and flu vaccination	1336/2662	Overall 0.94(0.64-1.10) Subgroup analysis 14-60 years: OR 0.53 (0.31- 0.91)	7
Knol et al (2008)	The Netherlands 1985-2003	Antipsychotic drug use and risk of pneumonia	Prescription data (e.g. Current use was defined as the most recent prescription ending within 7 days of the index date [date of hospital admission])	None	543/2706	1.30(1.10-1.60)‡	5
×Vergis, E. N. et al (2001)	US 1997-1998	Risk factors for the occurrence of pneumonia	Sedative use within 14 days prior to onset of pneumonia	admission date, level dependence, and duration of institutionalisation	104/208	2.60(1.20-5.40)	7

Author/ year	Location/ study period	Primary focus of the study	Definition of benzodiazepine use	Adjustment of confounders	Number of events*/ patients	Odds Ratio (95% CI)	Quality assessment score†
Almirall et al (1999)	Spain 1993-1995	Risk factors for CAP	Regular benzodiazepine use during the last year	Age, sex, municipality, weight, smoking status, any other respiratory infection, previous confirmed pneumonia, diabetes, chronic bronchitis, diagnosed asthma, Lung tuberculosis, chronic liver disease, aminophylline use and Use of aerosols and plastic pear-spacers	205/680	0.46(0.23-0.94)	5

Note: CAP=community acquired pneumonia; COPD=chronic obstructive pulmonary disease; CHF=chronic heart failure; FEV1=forced expiratory volume in 1 second; EF=ejection fraction, €all population based case-control studies, *pneumonia cases, †By the Newcastle Ottawa assessment Scale with a maximum score of 9, ‡Crude Odds Ratio, ˣ Not included in the meta-analysis

Figure 2.0.2 Forest plot



Appendix 3.0: Search terms based on Readcodes for identification of the main study diseases (asthma, ILI and pneumonia).

Appendix 3.1 Asthma Readcodes and search terms

Read code	Read term
H333.00	Acute exacerbation of asthma
H33..11	Bronchial asthma
H33z011	Severe asthma attack
8H2P.00	Emergency admission, asthma
H330.00	Extrinsic (atopic) asthma
H330.12	Childhood asthma
663V100	Mild asthma
663V200	Moderate asthma
663V000	Occasional asthma
H331.00	Intrinsic asthma
H33zz11	Exercise induced asthma
H330011	Hay fever with asthma
H330.13	Hay fever with asthma
H331.11	Late onset asthma
H33z000	Status asthmaticus NOS
H330111	Extrinsic asthma with asthma attack
H33z200	Late-onset asthma
H330000	Extrinsic asthma without status asthmaticus
663V300	Severe asthma
H312000	Chronic asthmatic bronchitis
H330z00	Extrinsic asthma NOS
H332.00	Mixed asthma
H334.00	Brittle asthma
173c.00	Occupational asthma
H331000	Intrinsic asthma without status asthmaticus

H331z00	Intrinsic asthma NOS
H330100	Extrinsic asthma with status asthmaticus
H331111	Intrinsic asthma with asthma attack
H331100	Intrinsic asthma with status asthmaticus
H35y700	Wood asthma
H35y600	Sequoiosis (red-cedar asthma)

Appendix 3.2 ILI Readcodes and search terms

Read code	Read term
H05z.11	Upper respiratory tract infection NOS
H051.00	Acute upper respiratory tract infection
H27..00	Influenza
H05z.12	Viral upper respiratory tract infection NOS
H27z.00	Influenza NOS
16L..00	Influenza-like symptoms
H27z.12	Influenza like illness
H054.00	Recurrent upper respiratory tract infection
H2A..11	Influenza A (H1N1) swine flu
H27y100	Influenza with gastrointestinal tract involvement
H271z00	Influenza with respiratory manifestations NOS
1J72.11	Suspected swine influenza
1J72.00	Suspected influenza A virus subtype H1N1 infection
H271100	Influenza with pharyngitis
H271000	Influenza with laryngitis
H271.00	Influenza with other respiratory manifestation
1W0..00	Possible influenza A virus H1N1 subtype
Hyu0700	[X]Influenza+other manifestations, virus not identified
H2A..00	Influenza due to Influenza A virus subtype H1N1
4JU0.00	Influenza H1 virus detected
H27y.00	Influenza with other manifestations
4J3L.00	Influenza A virus H1N1 subtype detected

Hyu0600	[X]Influenza+oth respiratory manifestatns,virus not identifd
H27yz00	Influenza with other manifestations NOS
4JU4.00	Influenza A virus, other or untyped strain detected
H27y000	Influenza with encephalopathy
4JU5.00	Influenza B virus detected

Appendix 3.3 Pneumonia Readcodes and search terms

Read code	Read term
H26..00	Pneumonia due to unspecified organism
H25..00	Bronchopneumonia due to unspecified organism
H21..00	Lobar (pneumococcal) pneumonia
H2...00	Pneumonia and influenza
H260.00	Lobar pneumonia due to unspecified organism
H261.00	Basal pneumonia due to unspecified organism
H28..00	Atypical pneumonia
H2y..00	Other specified pneumonia or influenza
H20..00	Viral pneumonia
H231.00	Pneumonia due to mycoplasma pneumonia
H22z.00	Bacterial pneumonia NOS
H2B..00	Community acquired pneumonia
H20z.00	Viral pneumonia NOS
SP13100	Other aspiration pneumonia as a complication of care
H22..00	Other bacterial pneumonia
H25..11	Chest infection - unspecified bronchopneumonia
H23..00	Pneumonia due to other specified organisms
H56y100	Interstitial pneumonia
H540000	Hypostatic pneumonia
H223.00	Pneumonia due to streptococcus
H2C..00	Hospital acquired pneumonia
H22y200	Pneumonia – Legionella
H270000	Influenza with bronchopneumonia

H22..11	Chest infection - other bacterial pneumonia
H224.00	Pneumonia due to staphylococcus
H540100	Hypostatic bronchopneumonia
H270.00	Influenza with pneumonia
H23z.00	Pneumonia due to specified organism NOS
H20..11	Chest infection - viral pneumonia
A3BXA00	Mycoplasma pneumoniae [PPLO] cause/dis classifd/oth chaptr
H22yz00	Pneumonia due to bacteria NOS
H201.00	Pneumonia due to respiratory syncytial virus
H220.00	Pneumonia due to klebsiella pneumonia
AB24.11	Pneumonia – candida
A3BXB00	Klebsiella pneumoniae/cause/disease classifd/oth chapters
H564.00	Bronchiolitis obliterans organising pneumonia
H060A00	Acute bronchitis due to mycoplasma pneumoniae
H20y.00	Viral pneumonia NEC
H221.00	Pneumonia due to pseudomonas
H24y200	Pneumonia with pneumocystis carinii
H240.00	Pneumonia with measles
Q310500	Congenital pneumonia due to viral agent
H270.11	Chest infection - influenza with pneumonia
H24y700	Pneumonia with varicella
H233.00	Chlamydial pneumonia
H243.00	Pneumonia with whooping cough
H24..00	Pneumonia with infectious diseases EC
H222.00	Pneumonia due to haemophilus influenza
Hyu0H00	[X]Other pneumonia, organism unspecified
H243.11	Pneumonia with pertussis
H22y.00	Pneumonia due to other specified bacteria
H564.11	Cryptogenic organising pneumonia
H21..11	Chest infection - pneumococcal pneumonia
A116.00	Tuberculous pneumonia

H530300	Abscess of lung with pneumonia
H23..11	Chest infection - pneumonia organism OS
H270z00	Influenza with pneumonia NOS
H246.00	Pneumonia with aspergillosis
4JUK.00	Mycoplasma pneumoniae detected
H24y000	Pneumonia with actinomycosis
H202.00	Pneumonia due to parainfluenza virus
H200.00	Pneumonia due to adenovirus
A3By400	Pleuropneumonia-like organism (PPLO) infection
H223000	Pneumonia due to streptococcus, group B
43n1.00	Mycoplasma pneumoniae antibody level
H471000	Lipoid pneumonia (exogenous)
Hyu0B00	[X]Pneumonia due to other specified infectious organisms
H222.11	Pneumonia due to haemophilus influenza
A730.00	Ornithosis with pneumonia
Hyu0A00	[X]Other bacterial pneumonia
4JRC.00	Atypical pneumonia screening test
A022200	Salmonella pneumonia
Q310200	Congenital pneumonia due to group B haemolytic streptococcus
Hyu0800	[X]Other viral pneumonia
A54x400	Herpes simplex pneumonia
H22y000	Pneumonia due to escherichia coli
H241.00	Pneumonia with cytomegalic inclusion disease
H24y.00	Pneumonia with other infectious diseases EC
43eG.00	Chlamydia pneumoniae IgG level
H247000	Pneumonia with candidiasis
Q310100	Congenital pneumonia due to group A haemolytic streptococcus
H242.00	Pneumonia with ornithosis
H571.00	Rheumatic pneumonia
H24y400	Pneumonia with salmonellosis
H22y011	E.coli pneumonia

H56y000	Endogenous lipoid pneumonia
H270100	Influenza with pneumonia, influenza virus identified
H24z.00	Pneumonia with infectious diseases EC NOS
A3C0300	Sepsis due to Streptococcus pneumonia
H24y300	Pneumonia with Q-fever
H24yz00	Pneumonia with other infectious diseases EC NOS
F00y400	Meningitis due to klebsiella pneumonia
H24y600	Pneumonia with typhoid fever
H22yX00	Pneumonia due to other aerobic gram-negative bacteria
H24y100	Pneumonia with nocardiasis
H232.00	Pneumonia due to pleuropneumonia like organisms
H22y100	Pneumonia due to proteus
Hyu0D00	[X]Pneumonia in viral diseases classified elsewhere
H247z00	Pneumonia with systemic mycosis NOS
AyuKA00	[X]Klebsiella pneumoniae/cause/disease classifd/oth chapters
H24y500	Pneumonia with toxoplasmosis
H247100	Pneumonia with coccidioidomycosis
AyuK900	[X]Mycoplasma pneumoniae [PPLO]cause/dis classifd/oth chaptr
H203.00	Pneumonia due to human metapneumovirus
H244.00	Pneumonia with tularaemia

Appendix 4.0: Search terms based on therapeutic codes for identification of study drugs (benzodiazepines and zopiclone)

Appendix 4.1 Benzodiazepines prodcodes

Prodcodes	Product name	Drug substance name
20	temazepam tablets 10mg	Temazepam
46	diazepam tablets 2mg	Diazepam
47	diazepam tablets 5mg	Diazepam
35	nitrazepam tablets 5mg	Nitrazepam

1729	temazepam tablets 20mg	Temazepam
1088	lorazepam tablets 1mg	Lorazepam
921	temazepam capsules 10mg	Temazepam
1400	diazepam tablets 10mg	Diazepam
1559	clonazepam tablets 500 micrograms	Clonazepam
2403	temazepam capsules 20mg	Temazepam
2122	chlordiazepoxide capsules 5mg	chlordiazepoxide hydrochloride
1463	chlordiazepoxide capsules 10mg	chlordiazepoxide hydrochloride
2091	lorazepam tablets 2.5mg	Lorazepam
780	temazepam oral solution 10mg/5ml	Temazepam
2073	clonazepam tablets 2mg	Clonazepam
4543	chlordiazepoxide tablets 5mg	chlordiazepoxide hydrochloride
3147	chlordiazepoxide tablets 10mg	chlordiazepoxide hydrochloride
2352	diazepam elixir 2mg/5ml	Diazepam
10650	RIVOTRIL tablets 0.5mg [ROCHE]	Clonazepam
6025	chlordiazepoxide hydrochloride tablets 5mg	chlordiazepoxide hydrochloride
7924	nitrazepam suspension 2.5mg/5ml	Nitrazepam
5294	chlordiazepoxide hydrochloride tablets 10mg	chlordiazepoxide hydrochloride
7391	midazolam injection 10mg/2ml	midazolam hydrochloride
3686	nitrazepam tablets 10mg	Nitrazepam
4176	diazepam rectal tubes 10mg	Diazepam
3205	diazepam capsules 5mg	Diazepam
7301	midazolam buccal solution 10mg/ml	midazolam hydrochloride
3234	midazolam injection 5mg/ml	midazolam hydrochloride

2083	DIAZEPAM RECTUBES rectal tubes 10mg [CP PHARM]	Diazepam
6747	diazepam rectal tubes 5mg	Diazepam
3870	diazepam capsules 2mg	Diazepam
8345	STESOLID rectal tubes 10mg [ACTAVIS]	Diazepam
10430	temazepam capsules 30mg	Temazepam
4395	STESOLID rectal tubes 5mg [ACTAVIS]	Diazepam
8029	RIVOTRIL tablets 2mg [ROCHE]	Clonazepam
7786	MOGADON tablets 5mg [ICN]	Nitrazepam
2407	nitrazepam capsules 5mg	Nitrazepam
10274	diazepam sugar free oral solution 2mg/5ml	Diazepam
7569	temazepam capsules 15mg	Temazepam
2078	DIAZEPAM RECTUBES rectal tubes 5mg [CP PHARM]	Diazepam
9430	diazepam suspension 2.5mg/5ml	Diazepam
4338	VALIUM tablets 5mg [ROCHE]	Diazepam
9696	alprazolam tablets 250micrograms	Alprazolam
14743	clonazepam suspension 500micrograms/5ml	Clonazepam
10802	XANAX tablets 250micrograms [PHARMACIA]	Alprazolam
13304	midazolam injection 5mg/5ml	midazolam hydrochloride
12849	diazepam suspension 10mg/5ml	Diazepam
11486	alprazolam tablets 500micrograms	Alprazolam
7392	midazolam injection 10mg/5ml	midazolam hydrochloride

12598	XANAX tablets 500micrograms [PHARMACIA]	Alprazolam
34002	TEMAZEPAM tablets 10mg [IVAX]	Temazepam
9814	nitrazepam suspension 5mg/5ml	Nitrazepam
8334	diazepam suppository 10mg	Diazepam
13279	lorazepam oral suspension 500mcg/5ml	Lorazepam
3524	MOGADON capsules 5mg [ROCHE]	Nitrazepam
8550	chlordiazepoxide tablets 25mg	chlordiazepoxide hydrochloride
2401	VALIUM tablets 2mg [ROCHE]	Diazepam
10278	EPISTATUS buccal solution 10mg/ml [SPEC PROD]	midazolam hydrochloride
13200	clonazepam sugar free oral solution 2mg/5ml	Clonazepam
17637	clonazepam sugar free solution 500micrograms/5ml	Clonazepam
8184	DIAZEPAM 10 MG INJ	
13305	midazolam injection 2mg/2ml	midazolam hydrochloride
5842	diazepam rectal tubes 2.5mg	Diazepam
8798	TEMAZEPAM GELTHIX gel-filled capsules 10mg [PHARMACIA]	Temazepam
34331	TEMAZEPAM tablets 10mg [HILLCROSS]	Temazepam
12124	diazepam injection (solution) 10mg/2ml	Diazepam
10954	ATIVAN tablets 1mg [WYETH PHAR]	Lorazepam
41516	TEMAZEPAM tablets 10mg [IVAX]	Temazepam
34482	DIAZEPAM tablets 5mg [TEVA]	Diazepam

5872	midazolam injection 2mg/ml	midazolam hydrochloride
10581	HYPNOVEL injection 10mg/2ml [ROCHE]	midazolam hydrochloride
39284	LORAZEPAM tablets 1mg [GENUS]	Lorazepam
34335	DIAZEPAM tablets 2mg [TEVA]	Diazepam
12477	LIBRIUM capsules 5mg [ICN]	chlordiazepoxide hydrochloride
9045	diazepam suspension 1mg/5ml	Diazepam
8913	LIBRIUM capsules 10mg [ICN]	chlordiazepoxide hydrochloride
41385	NITRAZEPAM tablets 5mg [ACTAVIS]	Nitrazepam
9065	diazepam oral solution 5mg/5ml	Diazepam
4483	clonazepam oral drops 2.5mg/ml	Clonazepam
5793	STESOLID rectal tubes 10mg [DUMEX]	Diazepam
7567	TEMAZEPAM PLANPAK capsules [???]	Temazepam
37745	lorazepam oral solution 1mg/5ml	Lorazepam
19450	MOGADON tablets 5mg [MEDA]	Nitrazepam
8344	diazepam suppository 5mg	Diazepam
37566	lorazepam oral suspension 1mg/5ml	Lorazepam
12462	TEMAZEPAM GELTHIX gel-filled capsules 20mg [PHARMACIA]	Temazepam
18488	DIAZEPAM RECTUBES rectal tubes 2.5mg [CP PHARM]	Diazepam
10402	VALIUM tablets 10mg [ROCHE]	Diazepam
6516	chlordiazepoxide hydrochloride tablets 25mg	chlordiazepoxide hydrochloride

6020	midazolam injection 1mg/ml	midazolam hydrochloride
9111	diazepam capsules 10mg	Diazepam
33648	TEMAZEPAM tablets 10mg [WYETH PHAR]	Temazepam
14417	LORAZEPAM 1 MG SUS	
664	lorazepam injection 4mg/1ml	Lorazepam
32296	DIAZEPAM tablets 5mg [ACTAVIS]	Diazepam
8842	DIAZEPAM RECTAL 2 MG/ML SOL	
34406	TEMAZEPAM tablets 10mg [TEVA]	Temazepam
816	clonazepam concentrate for solution for injection 1mg/1ml	Clonazepam
18125	LIBRIUM capsules 5mg [MEDA]	chlordiazepoxide hydrochloride
12237	diazepam injection (emulsion) 10mg/2ml	Diazepam
32500	clonazepam oral solution 250micrograms/5ml	Clonazepam
12278	FLURAZEPAM 15 MG TAB	
13756	DIAZEMULS injection 10mg/2ml [ACTAVIS]	Diazepam
4587	DIAZEPAM RECTAL 4 MG SOL	
20692	CHLORDIAZEPOXIDE	
12293	NORMISON capsules 10mg [WYETH PHAR]	Temazepam
30779	TEMAZEPAM tablets 20mg [WYETH PHAR]	Temazepam
9048	LIBRIUM tablets 5mg [ICN]	chlordiazepoxide hydrochloride
20701	CHLORDIAZEPOXIDE	
4439	LIBRAXIN tablets [ROCHE]	chlordiazepoxide/clidinium bromide
16610	VALIUM injection 5mg/ml [ROCHE]	Diazepam

20245	temazepam gel-filled capsules 10mg	Temazepam
24599	LIBRIUM capsules 10mg [MEDA]	chlordiazepoxide hydrochloride
17038	RIVOTRIL oral drops 2.5mg/ml [ROCHE]	Clonazepam
20801	TEMAZEPAM GELTHIX gel-filled capsules 30mg [PHARMACIA]	Temazepam
5856	HYPNOVEL injection 10mg/5ml [ROCHE]	midazolam hydrochloride
33672	DIAZEPAM tablets 2mg [HILLCROSS]	Diazepam
10409	LORAZEPAM .5 MG TAB	
34555	NITRAZEPAM tablets 5mg [WOCKHARDT]	Nitrazepam
34615	DIAZEPAM tablets 5mg [GEN (UK)]	Diazepam
15110	TEMAZEPAM sugar free elixir 10mg/5ml [GEN (UK)]	Temazepam
41607	DIAZEPAM tablets 10mg [TEVA]	Diazepam
34524	DIAZEPAM tablets 2mg [ACTAVIS]	Diazepam
17294	LIBRIUM tablets 10mg [ICN]	chlordiazepoxide hydrochloride
34408	NITRAZEPAM tablets 5mg [HILLCROSS]	Nitrazepam
34807	DIAZEPAM tablets 10mg [ACTAVIS]	Diazepam

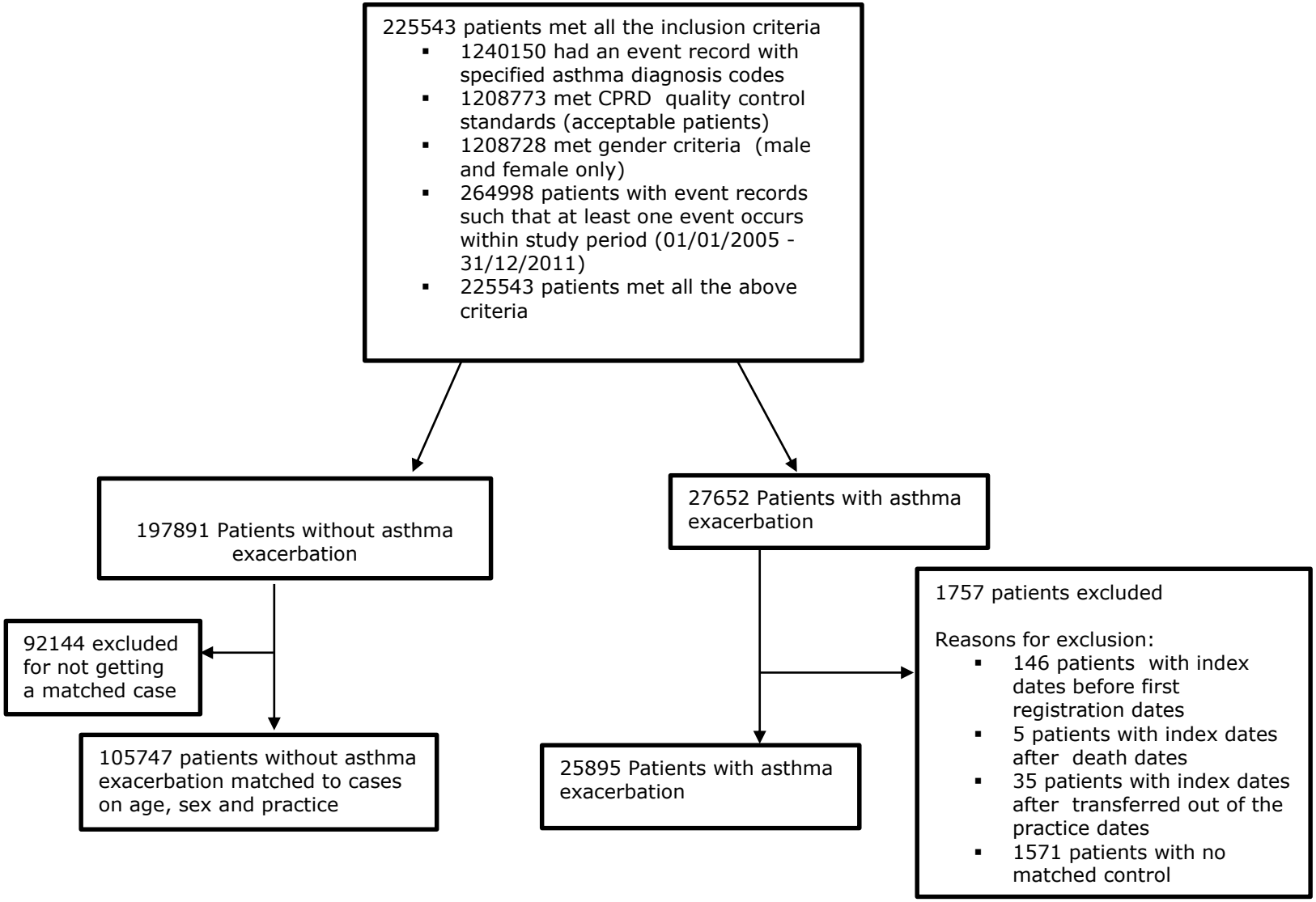
Appendix 4.2 Zopiclone prodcodes and search terms

Prodcode	Product name	Drug substance name
66	zopiclone tablets 7.5mg	Zopiclone
721	zopiclone tablets 3.75mg	Zopiclone
3320	ZIMOVANE tablets 7.5mg [AVENTIS]	Zopiclone
4187	ZIMOVANE LS tablets 3.75mg [AVENTIS]	Zopiclone
5058	ZILEZE tablets 3.75mg [OPUS]	Zopiclone

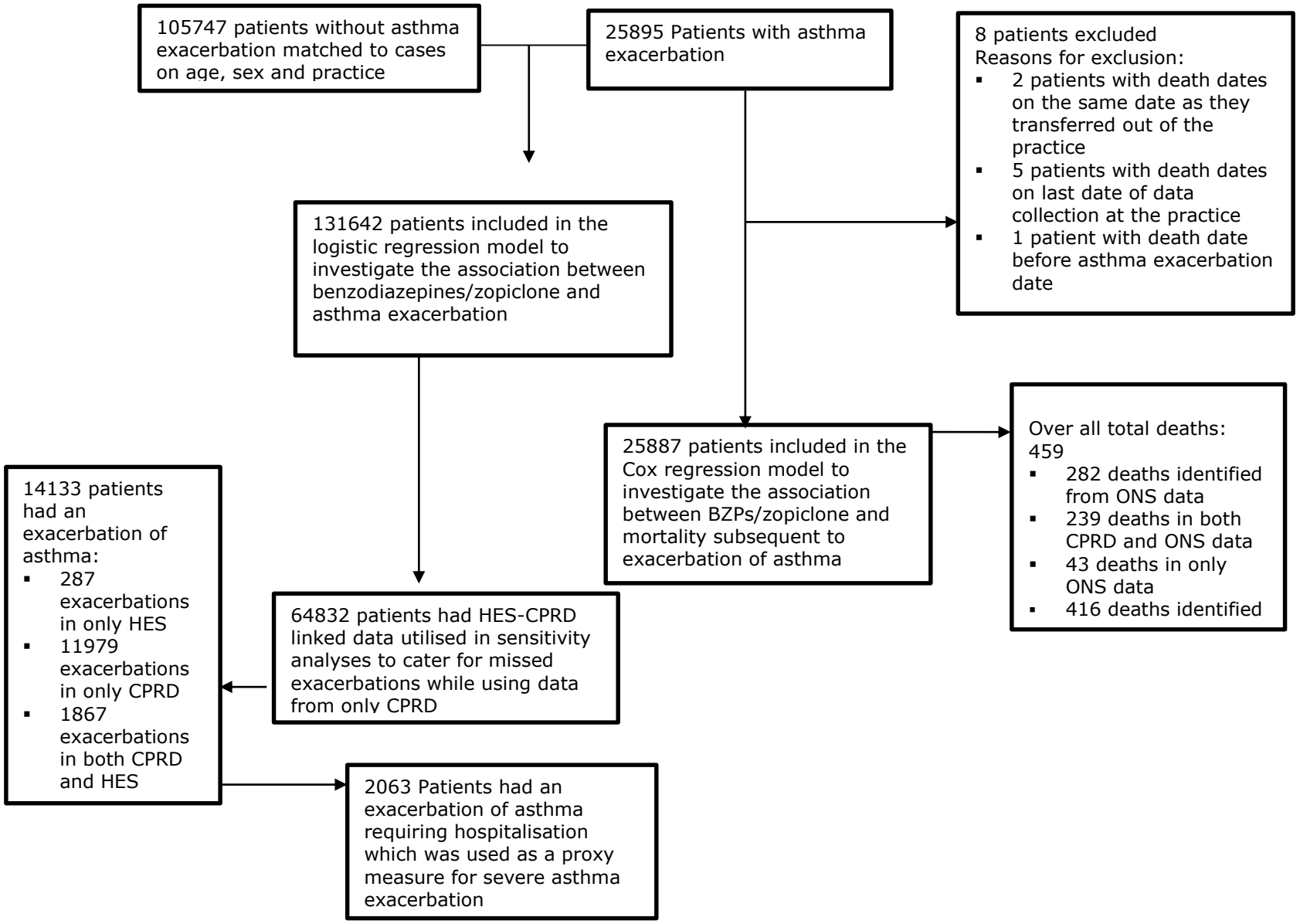
14365	zopiclone oral suspension 3.75mg/5ml	Zopiclone
15852	ZILEZE tablets 7.5mg [OPUS]	Zopiclone
24135	zopiclone tablets 7.5mg [ACTAVIS]	Zopiclone
29219	Zopiclone tablets 3.75mg [ACTAVIS]	Zopiclone
30056	Zopiclone tablets 3.75mg [IVAX]	Zopiclone
30377	Zopiclone tablets 3.75mg [GEN (UK)]	Zopiclone
33045	Zopiclone tablets 7.5mg [IVAX]	Zopiclone
33663	Zopiclone tablets 7.5mg [GEN (UK)]	Zopiclone
34372	Zopiclone tablets 7.5mg [PLIVA]	Zopiclone
34612	Zopiclone tablets 3.75mg [HILLCROSS]	Zopiclone
34777	Zopiclone tablets 3.75mg [TEVA]	Zopiclone
34823	Zopiclone tablets 7.5mg [TEVA]	Zopiclone
34874	Zopiclone tablets 7.5mg [KENT]	Zopiclone
34897	Zopiclone tablets 3.75mg [KENT]	Zopiclone
43445	Zopiclone tablets 7.5mg [HILLCROSS]	Zopiclone
45353	Zopiclone tablets 7.5mg [SANDOZ]	Zopiclone
46799	zopiclone oral solution 3.75mg/5ml	Zopiclone

Appendix 5.0: Flow charts summarising the selection strategy for study subjects

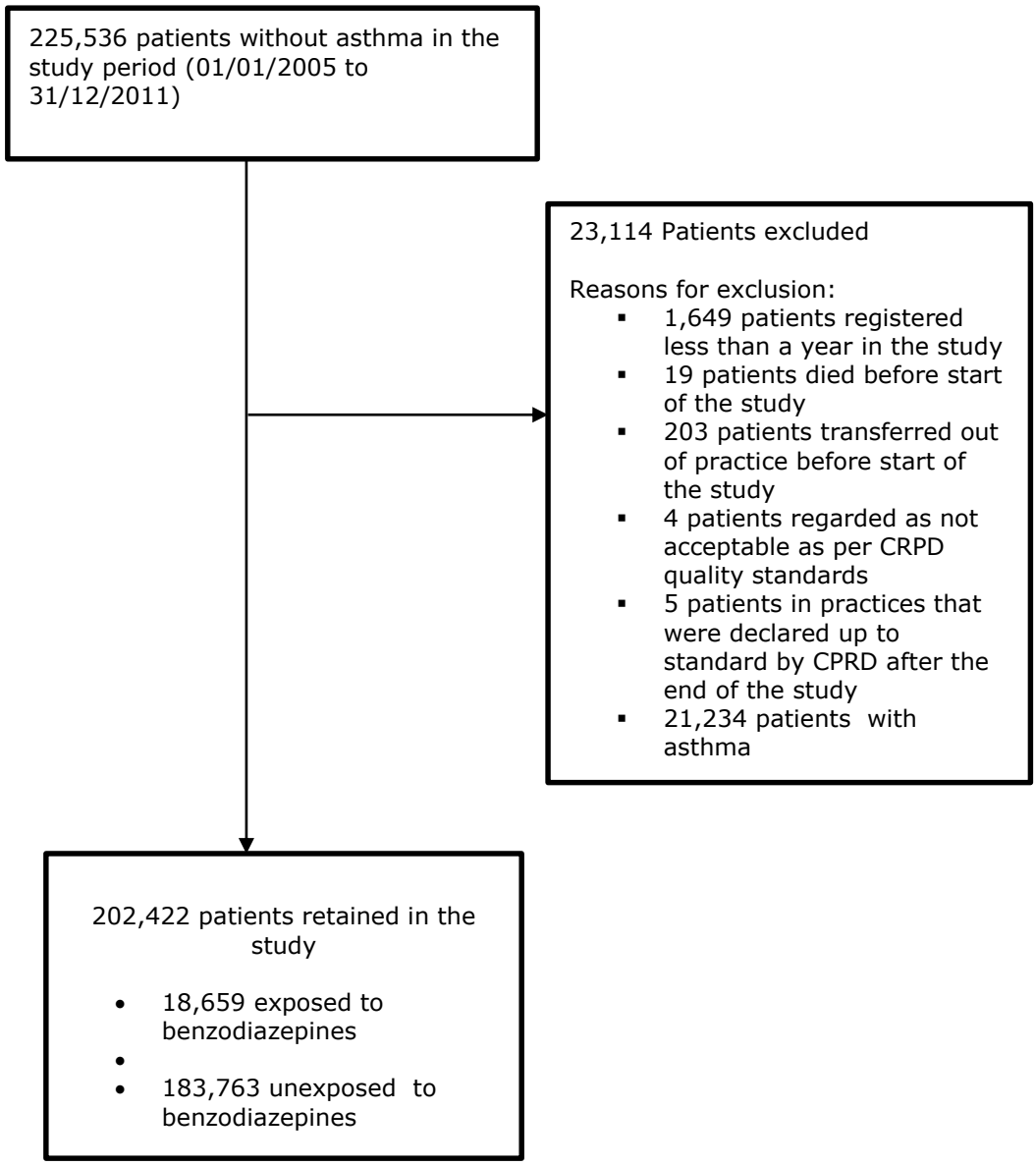
Appendix 5.1 Asthma study subject selection procedure



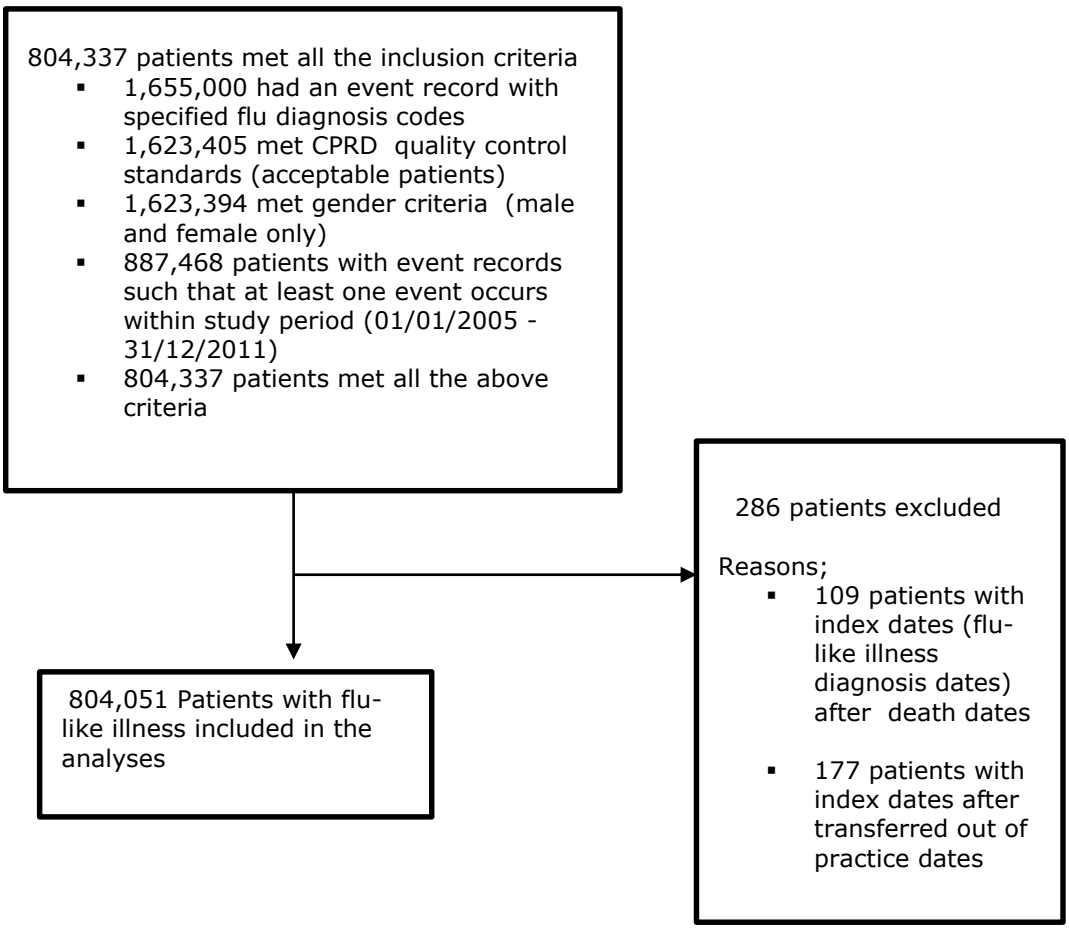
Appendix 5.1 continued



Appendix 5.2 Non-asthma study subject selection procedure



Appendix 5.3 ILI study subject selection procedure



Appendix 6.0: Asthma study publication

Nakafero G, Sanders RD, Nguyen-Van-Tam JS, Myles PR. Association between benzodiazepine use and exacerbations and mortality in patients with asthma: a matched case-control and survival analysis using the United Kingdom Clinical Practice Research Datalink. *Pharmacoepidemiology and Drug Safety* 2015

Appendix 7.0: Sample size calculations

1. Nested case-control study					
Outcome measure		Probability of benzodiazepine use in controls	Odds ratio (cases v. Controls)	Cases needed	Controls needed
Asthma study					
Outcome measure	Asthma exacerbation	0.17[1]	2.00[2]	119	714
2. Cohort study					
Outcome measure		Ratio of unexposed to exposed	Hazard ratio (unexposed v. Exposed)	Exposed patients	Unexposed patients
Asthma study					
Outcome measure	30-day mortality	3	1.2	718	2154
ILI study					
Outcome measure	Pneumonia	3	1.5	160	480
	30-day mortality	3	1.3	358	1074

Notes:

a. Calculated using the Dupont Power and Sample size program as described in Dupont WD, Plummer WD: "Power and Sample Size Calculations: A Review and Computer Program", Controlled Clinical Trials 1990; 11:116-28.

b. Standard assumptions:

Powered at 80% with a type 1 error probability of 0.05, ratio of control to cases 6:1

Correlation coefficient for exposure between matched cases and controls assumed to be 0.2 based on recommendations in Dupont (1988)

Median survival time in unexposed cases assumed to be 6 years (based on previous work in this topic area) with the ratio of unexposed to exposed subjects 3:1

[1] Probability of benzodiazepine use in controls based on population prevalence derived from THIN

[2] Based on previous point estimates obtained when investigating the association between benzodiazepine use and pneumonia incidence in THIN

Appendix 8.0: Reviewers comments on the asthma study manuscript submitted to Lancet Respiratory Medicine Journal that led to the non-asthma study

Reviewer #1: Major Concerns:

1. The authors mention that psychiatric disease as a confounder was accounted for, but more details are needed. This is an obvious confounder and there links between anxiety and asthma and psychiatric disease and mortality. This analysis needs careful analysis to separate these from asthma and death from all causes. What is the analysis results with and without accounting for these particular confounders?

2. The authors show an increase in "all cause" mortality. What about an increase in the death from asthma? Could "all cause" mortality be due to the presence of confounding psychiatric disease for which the patient is taking benzodiazepines?

3. Table 1 provides data on patients studied with asthma, why are patients without asthma not shown?

4. Most critical point: If there is an increase in risk of death of asthmatics taking benzos, what about non-asthmatics taking benzos? This is a very important measurement that would give needed strength to the study. If both populations show an increase, it would suggest that the mechanism of the increased death rate is not linked to GABAergic signaling in asthma.

5. If the proposed mechanism of a benzo effect on asthma excerbations or death rate is the interaction of benzos with GABAA receptors in lung relevant cells, why are there differences not found with all the benzos studied?

6. Results: "We estimated that over a six month follow-up period, the number needed to harm was 27 (95% CI 15, 191), indicating that for every 27 asthma patients prescribed benzodiazepines, one additional asthma exacerbation could be expected within six months." This seems like a clinically insignificant small increase.

7. Page 9 discussion: The phrase "that benzodiazepines were found to increase the occurrence of and mortality from asthma exacerbation" implies that death from asthma was measured when the manuscript seems to state that only "all-cause" mortality was measured. This is a very important distinction and limitation of the study since a measure from death from asthma associated with benzo use would greatly strengthen the conclusions of the study.

8. Discussion: I do not think that the authors findings support this statement: "However, the results support the previously observed association of benzodiazepines with an increased risk of pneumonia and pneumonia-related mortality in a study by Obiora et al.11" Asthma exacerbations are not universally due to immune cell alteration in function and even when it is, immune cells involved in the inflammatory response of asthma are quite different than those involved in infection.

Minor Concerns

1. Introduction page 3: "Expression of benzodiazepine-sensitive GABAA receptors is also increased on alveolar macrophages with IL-4 treatment ex vivo or allergen-induced asthma by house dust mite treatment in vivo.10" What is the reference for this statement referring to house dust mite antigen? It is not included in reference 10.

2. Appropriate references should be included for these two phrases in the discussion: "(i) augmenting benzodiazepine-sensitive GABAA receptor signaling during infection increases mortality from infection via impairing bacterial clearance, and (ii) that allergen-induced airway disease leads to an up regulation of GABAA receptor expression on macrophage and pulmonary epithelium."

Reviewer #2: BNZ_ case control

Title and abstract:

1. Mention study setting as well in your title.

2. Just write nested case control study, delete cohort from the title.

3. Mention adjusted matched odds ratios (mOD adj) I suggest you not to use the term risk estimation for case control design.

Introduction:

1. In general your introduction is fine, however I recommend you to start with the burden of asthma worldwide and region wide magnitude.

2. Do you have any data on prevalence i.e. use of benzodiazepines among asthmatic? Cite Gaps in the literature are not highlighted appropriately. Work on this further.

3. You need to highlight further why this association study is warranted. Work on this.

4. I suggest you to write hypothesis as well "we hypothesize that among asthmatic patients using BNZ exacerbation is greater than controls.

Methods:

1. Clarity in the case definition is missing; you need to work on how the cases were identified Similarly recruitment of controls is also unclear. Were they selected by density sampling?

2. What was the rationale for matching on general practice, elaborate?

3. Were age and gender confounders, if yes mentioning this?

4. You did assess for compliance as it was a linked data, this is one of the limitation.

5. Did you gather data of doses and frequency?

6. Polypharmacy is one the major confounder that requires adjustment; besides this nutritional and physical activity data also need consideration.

7. Was there any sample size estimation?

8. For analysis mention descriptive analysis, also mention about making matched pairs for each case with 6 controls.

9. In inferential analysis for continuous variables did you check for their linearity?

Results:

1. First mention the descriptive analysis.

2. How the data on smoking and alcohol consumption was gathered. Elaborate.

3. Which method was used for estimation of PAF for case control, there are number of methods, please cite.

4. The estimation of PAF is ideal for population based studies. Comment.

5. Generalizability is a major concern for PAF estimation, please comment.

6. Causality is important for the interpretation of the PAF. Hence, to label any risk factor as associated with the disease, there should be a cause-and-effect relationship. Then one can only say that by removing the risk of a particular exposure from the population, we can prevent the incidence of further cases. Comment.

7. How the data on depression and psychosis was collected, was it by validated tool? Was psychiatrist involved?

8. the write up require furhter modifications.

Reviewer #3: This is a well written paper investigating the relationship between asthma exacerbation and mortality. The introduction is succinct and the hypothesis clear.

Specific points:

1. There is no adjustment for asthma severity. This may confound results.

2. There is likely to be a significant difference for other reasons between asthma patients who exacerbate and those who do not. This should be discussed.

3. Data are available on cause of death and proportion of respiratory deaths should be investigated. The authors state in the introduction that they are interested in asthma related mortality but then look at all cause mortality only.

4. Asthma exacerbation codes chosen do not include any infection codes and are likely to be missing exacerbations.

5. Severity of asthma exacerbation is not accounted for. E.g hospitalisation. Again these exacerbations have potentially been missed. Those seen in hospital or A&E only may not be captured.

6. Adjusting for use of statins and oral corticosteroids at any time prior to the index date is not entirely logical and if only one prescription was ever given and that was several years prior to the index date, it is not clear how that was dealt with.

7. Corticosteroid use could have indicated asthma exacerbation.

8. Some indication of the proportion of missing data should be provided.

9. The authors state that ".....variations in coding practices in different general practices could have led to misclassification....." but make no attempt to do sensitivity analyses addressing possible errors. For example, there is no comment on use of steroids or antibiotics as a proxy for asthma exacerbation.

10. The presence of only 1 asthma code was required for inclusion into the cohort, while use of 2 may introduce an immortal time bias, use of one code only could lead to significant over diagnosis of asthma, particularly in the controls.

11. The tables do not require a column for p values

12. The temporality of the association is not clear and the reason for prescribing the benzodiazepines in the first place may actually be the reason for increased mortality and not the drugs themselves.

13. To investigate whether benzodiazepines increase exacerbation risk in asthma, methodologically it may have made more sense to have done a SCCS.

Appendix 9.0: Third year annual review: Approval of the five PhD studies by internal examiner

The University of Nottingham
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School Research Student Annual Review Form (excluding Probationary PhD students)

This form must be completed as part of the annual review of the progress of research students and lodged in the student's School/Divisional file where it must be available for monitoring and appeals purpose. Full details of the Annual Review process are in the Research Degrees section of the Quality Manual.

Section One – Student/Supervisor Details

Student ID: **4154645** Name of student: **Nakafero**

Date of initial registration: **01/10/2011** Year of study: **3rd year**

Current registration (PhD/MPhil/Other): **PhD** Mode of study (FT/PT): **Full time**

Title of project: **The association of benzodiazepines and occurrence of asthma exacerbation, influenza-like illness related pneumonia and mortality.**

Main supervisor (name/School/Division): **Dr. Puja Myles; Division of Epidemiology and Public Health, School of Medicine**

Additional supervisor(s)(name/School/Division): **Prof. Jonathan Nguyen Van-Tam; Division of Epidemiology and Public Health, School of Medicine**

Section Two – Internal Assessor Report (if applicable)
Please comment specifically on each element of the annual review.
Internal Assessor(s)(name/School/Division): **Dr Elizabeth Orton, Division of Primary Care, School of Medicine**

Internal assessor's report Georgina Nakafero –third year report

Georgina's thesis is a series of 5 studies on benzodiazepine use and risk of asthma exacerbations, asthma mortality, all cause mortality, Ill-related mortality and Ill-related pneumonia. This year she has finished all of her analyses and written up the methods and results tables. She has two papers that have been submitted to journals but need revising and this has been put on hold temporarily whilst she finishes her thesis write-up. Georgina will progress into her thesis pending year on the 1 October 2014 and plans to submit her thesis at the end of September 2015. She seems to be on course for this. However, I have asked Georgina to give me an informal update at the end of January 2015 regarding her progress writing. This is just precautionary and I do not foresee any problems. She is writing up away from the university campus but has access to all of the facilities she needs to do this (computer, internet, papers, books etc) and has a planned programme of submitting chapter drafts to her supervisors for comment throughout the year.
Based on this, I am happy to recommend that Georgina progresses into the thesis pending year from 1 October 14.