The role of influenza and acute respiratory infections as triggers for acute myocardial infarction

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Declaration

I, Charlotte Michelle Jane Warren-Gash confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Candidate's signature

Abstract

Background: Influenza is an important global cause of morbidity and mortality. Though some cardiac complications of influenza, such as myocarditis, are well-recognised, its role as a trigger for acute cardiovascular events is less clear. Improved understanding of this relationship will add to evidence to support appropriate prevention and treatment strategies.

Methods: I investigated evidence that influenza and acute respiratory infections could trigger acute myocardial infarction (AMI) through a systematic literature review and meta-analysis (chapter 2) and original research studies (chapters 3-7). These were an ecological weekly time series study using Poisson regression models adjusted for temporal and environmental confounders in England & Wales and Hong Kong (chapter 3); two self-controlled case series analyses using the General Practice Research Database linked to influenza surveillance data (chapter 4) and to cardiac disease registry and hospitalisation data (chapter 5); a case control study in AMI patients and surgical controls during the 2009 influenza pandemic in London (chapter 6); an exploratory mechanistic study (chapter 7).

Key findings:

- Acute respiratory infections, and seasonal influenza, triggered AMI
- A triggering effect may be greater for influenza than for other respiratory infections (p=0.011)
- AMI risk was highest in the first three days after acute infection – adjusted incidence ratio 4.19 (95% CI 3.18-5.53) – and persisted for around 28 days
- The proportion of AMI deaths due to seasonal influenza ranged from 3-5%, rising to 13% in periods of highest influenza circulation
- The relative risk of AMI after acute respiratory infection was highest in people aged ≥80 years
- Influenza vaccination protected against some adverse cardiac outcomes in people with existing cardiovascular disease

Conclusions: Reducing the burden of influenza would benefit cardiovascular health. Questions remain about key groups to target, as well as the optimal type and delivery of interventions to reduce influenza-associated AMI risk.

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List of abbreviations

ACS – Acute coronary syndrome

AIC – Akaike information criterion

AMI – Acute myocardial infarction

ARI – Acute respiratory infection

CABG – Coronary artery bypass graft

CALIBER – CArdiovascular disease research using Linked Bespoke studies & Electronic Records

CfI – Centre for Infections

CHD – Coronary heart disease

CI – Confidence interval

CRP – C-reactive protein

CVD – Cardiovascular disease

ECG – Electrocardiogram

ELISA – Enzyme-linked immunosorbent assay

GPRD – General Practice Research Database

HES – Hospital Episode Statistics

HI – Haemagglutinin inhibition

HPA – Health Protection Agency

HR – Hazard ratio

ICD – International Classification of Diseases

ILI – Influenza-like illness

IL-6 – Interleukin-6

IQR – Interquartile range

IR – Incidence ratio

MINAP – Myocardial Ischaemia National Audit Project

NICE – National Institute for Health and Clinical Excellence

NSTEMI – Non ST segment elevation myocardial infarction

OR – Odds ratio

PCI – Percutaneous coronary intervention

RCGP – Royal College of General Practitioners

RCT – Randomised controlled trial

RH – Relative humidity

RR – Risk ratio or rate ratio (defined as needed)

RT-PCR – Reverse transcriptase polymerase chain reaction

SAA – Serum amyloid A

SIGN – Scottish Intercollegiate Guidelines Network

STEMI – ST segment elevation myocardial infarction

TNF-α – Tumour necrosis factor-α

URI – Upper respiratory infection

VWF – Von Willebrand factor

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1. Key features of influenza and acute myocardial infarction relevant to the research aims

1.1 Description of chapter contents

In this chapter I outline the background to the hypothesis that acute respiratory infections such as influenza can trigger acute myocardial infarction (AMI). Seasonality, epidemiology, clinical characteristics, diagnosis and control of influenza are described with an emphasis on cardiac complications. The focus is on aspects of influenza relevant to the research rather than on providing a comprehensive overview of influenza. The epidemiology of AMI is described including an introduction to 'traditional' vascular risk factors and their potential interaction with triggers of atherosclerotic plaque instability such as infections. Key points at which a relationship with influenza might be relevant for AMI management are highlighted. The chapter ends with an overview of the thesis aims and objectives.

1.2 Infection as a trigger for acute myocardial infarction

1.2.1 Introduction to hypothesis

Various socio-demographic, lifestyle and clinical risk factors for cardiovascular disease have been well-documented over recent decades[1–3]. Nevertheless people without these traditional factors continue to experience AMI, which has prompted a search for novel risk factors[4]. A relationship between infection and cardiovascular disease was first proposed over a hundred years ago; William Osler writing in 1908 suggested that acute infections were an important factor in the 'causation' of atherosclerosis[5]. As atherosclerosis was considered to be a disorder primarily of lipid storage until the 1970s[6], links with infection seemed biologically implausible. Now there is widespread recognition of the central role played by inflammation in the pathogenesis of ischaemic heart disease at all stages from early atherogenesis to downstream thrombotic events[7]. Infections such as influenza act as acute inflammatory stimuli[8] so may plausibly interact

with ongoing systemic inflammatory processes to trigger acute cardiovascular events. The increase in both influenza and AMI incidence during cold winter months combined with the recognition of cardiac complications among influenza patients stimulated interest in influenza infection as a potential AMI trigger.

1.2.2 Importance

Ischaemic heart disease, predominantly due to AMI, is the world's leading cause of death. It is projected to remain so in 2020[9]: the decline in incidence and case fatality of AMI in many developed countries over recent decades[10,11] is countered by the epidemiological transition occurring in some developing nations as well as by population ageing, rising obesity levels and persisting health inequalities. Each year a large number of non-respiratory deaths are attributed to influenza, for example from cardiovascular causes, and this merits further explanation. If a causal association were demonstrated between influenza and AMI, this could have implications for vaccine and antiviral policies as well as on influenza pandemic preparedness. The 2009 influenza A H1N1 pandemic was a timely reminder of the clinical and public health importance of this area and the need for informed policy and emergency planning decisions to be taken on a firm evidence base.

1.3 Influenza epidemiology, clinical features, diagnosis & control

1.3.1 Seasonality

Seasonal epidemics of human influenza virus infection are caused either by influenza A (divided into subtypes H3N2 or H1N1 based on the composition of the two main surface glycoproteins haemagglutinin and neuraminidase) or influenza B[12]. In temperate climates, outbreaks tend to be dominated by type A viruses with type B following later in the season[13], which typically lasts from around November until March. Epidemics recur annually because antigenic variation (or 'drift') in viral surface glycoproteins allows people who have been infected previously to become susceptible[14]. In sub-tropical zones such as Hong Kong two influenza seasons rather than one have been reported and in some

equatorial regions such as Singapore viruses have been isolated all year round[15]. An influenza pandemic, characterised by worldwide spread of a previously unknown virus, may occur outside of the usual circulation period for influenza. It usually requires antigenic shift in influenza A virus, which may result from genetic reassortment between several subtypes of influenza affecting one host, to produce a novel antigenic variant[16].

1.3.2 Clinical characteristics

Estimates of the proportion of asymptomatic seasonal influenza infections vary widely from around 25-75% of infections[17,18], although figures of 33-50% are typically used for mathematical modelling studies[19]. Symptomatic infection is characterised by an abrupt onset of feverish illness associated with respiratory tract symptoms such as cough and sore throat as well as systemic features such as myalgia and fatigue. While most cases of influenza are mild and self-limiting, complications such as secondary bacterial infections eg pneumonia or otitis media occur in a small proportion of people. These are usually people at the extremes of age, pregnant women or those with chronic medical conditions[20]. The likelihood of residual cross-immunity from previous influenza infections is directly proportional to age (so attack rates are generally highest in children)[21]. Although children and the elderly have the highest rates of hospitalisations due to influenza, mortality from seasonal influenza in people aged over 60 years is approximately 10 times higher than in young children[22]. Deaths and more severe complications tend to be associated with the influenza A H3N2 subtype[23], while influenza B viruses sometimes produce clusters of unusual conditions such as myositis[24].

In a pandemic situation, profiles of those affected can change dramatically as there is little pre-existing immunity in a population. For example in the 1918 influenza A H1N1 'Spanish flu' pandemic an unusually high case fatality was seen in young adults[25]. People of a similar age distribution were affected, though much less severely, by the 2009 influenza A H1N1 pandemic. In the other two modern day pandemics of 1957 and 1968, patterns of mortality were found to resemble more closely those seen with seasonal influenza[25,26].

1.3.3 Cardiac effects

In acute influenza infection, cardiac pathology is thought to result either from direct effects of the virus on the myocardium or through exacerbation of underlying cardiovascular disease[27]. Influenza-associated myocarditis, characterised by focal infiltration of inflammatory cells, interstitial oedema and cardiac necrosis, may be present in up to 10% of patients infected with influenza [28]. It is, however, frequently undiagnosed and may be masked by respiratory symptoms. While the majority of cases are mild and resolve spontaneously, influenza-associated myocarditis can precipitate congestive cardiac failure and even death in some cases[28]. The fact that excess hospital admissions and deaths occur during the influenza season due to cardiovascular (rather than respiratory) causes is also well documented[29,30]. New cardiac arrhythmias, incipient cardiac failure and acute coronary syndromes have been demonstrated in studies of patients with community-acquired pneumonia[31]. Nonetheless the relationship between influenza infection and AMI is less clear and provides the focus for the following systematic literature review (chapter 2) and research studies (chapters 3-7). Though other acute cardiovascular events such as thrombotic stroke or acute limb ischaemia might also be triggered by influenza this thesis focussed on AMI, to allow in-depth study of one important outcome.

1.3.4 Diagnosis and treatment

The majority of influenza cases are not formally diagnosed, either due to their asymptomatic nature or failure to seek medical attention. Patients who do present to primary care are most likely to be diagnosed using a clinical algorithm. For example general practitioners would have a high index of suspicion if a patient presented with an influenza-like illness (ILI – defined by the World Health Organization as acute fever>38˚C and cough or sore throat in the absence of another diagnosis) during time periods when influenza is circulating. This definition has modest sensitivity (around 65%) and specificity (67%) for diagnosing true influenza infections[32]. Clinical symptoms of influenza are mimicked by many other respiratory viruses including rhinovirus, coronavirus,

respiratory syncytial virus and parainfluenza virus[33]. The positive predictive value of an ILI diagnosis varies markedly depending on prevalence of circulating influenza, and can be up to 70-80% at times of peak circulation[34]. Other factors affecting the accuracy of ILI for diagnosing influenza are discussed in chapter 6, section 6.2.4, p153. Laboratory testing of respiratory or serum specimens tends to be done on hospitalised cases and is commoner as symptom severity increases or complications develop. It is described in chapter 6, sections 6.2.5-6, p153-154.

Most treatment for influenza is supportive, involving for example rest, rehydration and symptomatic relief such as use of antipyretics. Antiviral drugs (which may be of two classes – neuraminidase inhibitors such as oseltamivir and adamantanes such as amantadine[12]) – tend to be reserved for patients at high risk of complications from seasonal influenza. In a pandemic situation they may be used more widely for both prophylaxis and treatment to limit transmission.

1.3.5 Impact on population morbidity and mortality

In England & Wales estimates from mathematical models suggest that there are 18,500-24,800 deaths, 19,000-31,200 hospital admissions and 779,000-1,164,000 general practice consultations attributable to seasonal influenza each year[35]. Worldwide, there may be up to 3 to 5 million cases of severe illness and 250,000 to 500,000 deaths annually[12], mainly among high risk groups. Direct costs to health services (estimated as $10.4billion USD annually in the USA[36]) represent only part of the economic impact of influenza. Loss of life and days of work or school lost to illness lead to substantial indirect costs through projected loss of earnings (amounting to an average of $16.3 billion USD annually in the USA[36]). The impact of influenza is due in part to its global nature: it affects all age groups, occurs worldwide and recurs in individuals[37]. The effect of a pandemic is more variable depending on strain type and illness severity but the potential for overwhelming of clinical services, large numbers of deaths and widespread disruption to services and infrastructure necessitates contingency planning at local, national and international levels.

1.3.6 Transmission and prevention

Influenza is a highly contagious disease[37]. Transmission occurs through several routes including direct physical transfer of micro-organisms from infected individuals to others, indirect transfer via an intermediate object such as contaminated hands or surfaces, transmission via large droplets (≥5μm diameter) generated through coughing, sneezing or talking and airborne transmission via small aerosolised particles[38]. Interventions to prevent or reduce influenza spread may be non-pharmaceutical or pharmaceutical such as vaccine and antiviral drugs. Non-pharmaceutical measures, which include hand and respiratory hygiene, facemask use, social distancing, school closures and screening at entry ports, will not be discussed further here.

Influenza vaccination (using trivalent inactivated vaccine or live attenuated influenza vaccine) is currently an effective way to prevent disease and reduce the incidence of severe complications. A new vaccine is formulated every year whose composition is based upon the three most representative influenza strains circulating in humans identified through the World Health Organization (WHO) Global Influenza Surveillance Network[12]. Vaccine effectiveness ranges from 60 to 80% in healthy adults[39] and is highest when circulating strains are well-matched with those in the vaccine. Protection against infection is lower in the elderly but immunisation has been shown to reduce the incidence of severe complications, hospitalisations and deaths in this group[40]. In England, the Department of Health recommends that for the 2012/13 influenza season vaccination is offered to the following groups: all people aged 65 years and over; patients aged six months or older with chronic respiratory, cardiac, renal, liver or neurological disease, diabetes or immunosuppression, all pregnant women, all residents of long-stay residential care homes, carers and health and social care workers[40].

1.3.7 Current relevant issues in vaccine research and policy

Active areas of influenza vaccine research include the search for the 'holy grail' of a universal influenza vaccine that provides lasting protection against all strains as well as finding optimum methods to formulate and deliver existing vaccines eg

intra-nasally rather than by needle-based injection. Aside from debates about vaccine effectiveness, influenza vaccine uptake in people with chronic diseases in England tends to be low – it was 51.6% in the 2011/12 influenza season[41] – limiting its potential to prevent influenza and its complications. The Department of Health has set an ambitious target of increasing vaccine uptake in people aged under 65 years with chronic diseases to 75% by 2013/14[42]. It is hoped that vulnerable unvaccinated people in the community will also be protected indirectly by the introduction of a vaccination programme for healthy children in England from 2014, following recommendations by the Joint Committee on Vaccination and Immunisation (an expert advisory committee to the Government). The evidence that influenza vaccine may offer protection against acute cardiovascular events (hypothesised to occur either directly through reducing the risk of influenza, or indirectly through reducing influenza complications such as dehydration or bacterial super-infection[43]) will be critically reviewed in chapter 2.

1.4 Acute myocardial infarction epidemiology, risk factors, definition & management

1.4.1 Epidemiology

Acute myocardial infarction is a major cause of morbidity and mortality worldwide, with >7 million people experiencing an AMI each year[44]. In general AMI incidence increases with age and it is more common in men. Substantial geographic variation is found in both age and gender distributions of AMI: in the global INTERHEART study the median age of onset of first AMI was 51 years in the Middle East and 63 years in China; the proportion of male cases ranged from 68% in Central and Eastern Europe to 85% in South Asia[3]. Around 80% of the global burden of cardiovascular disease occurs in low- and middle-income countries where healthcare infrastructures are less developed[3]. Progressive urbanisation in these settings has led to increasing rates of obesity and diabetes and an emerging epidemic of atherosclerotic coronary artery disease[44]. Any intervention to prevent influenza that indirectly protects against AMI might

feasibly have a greater impact in the developing world where access to cardiac catheter laboratories and specialist cardiac services is limited.

1.4.2 Risk factors for atherosclerosis and acute myocardial infarction

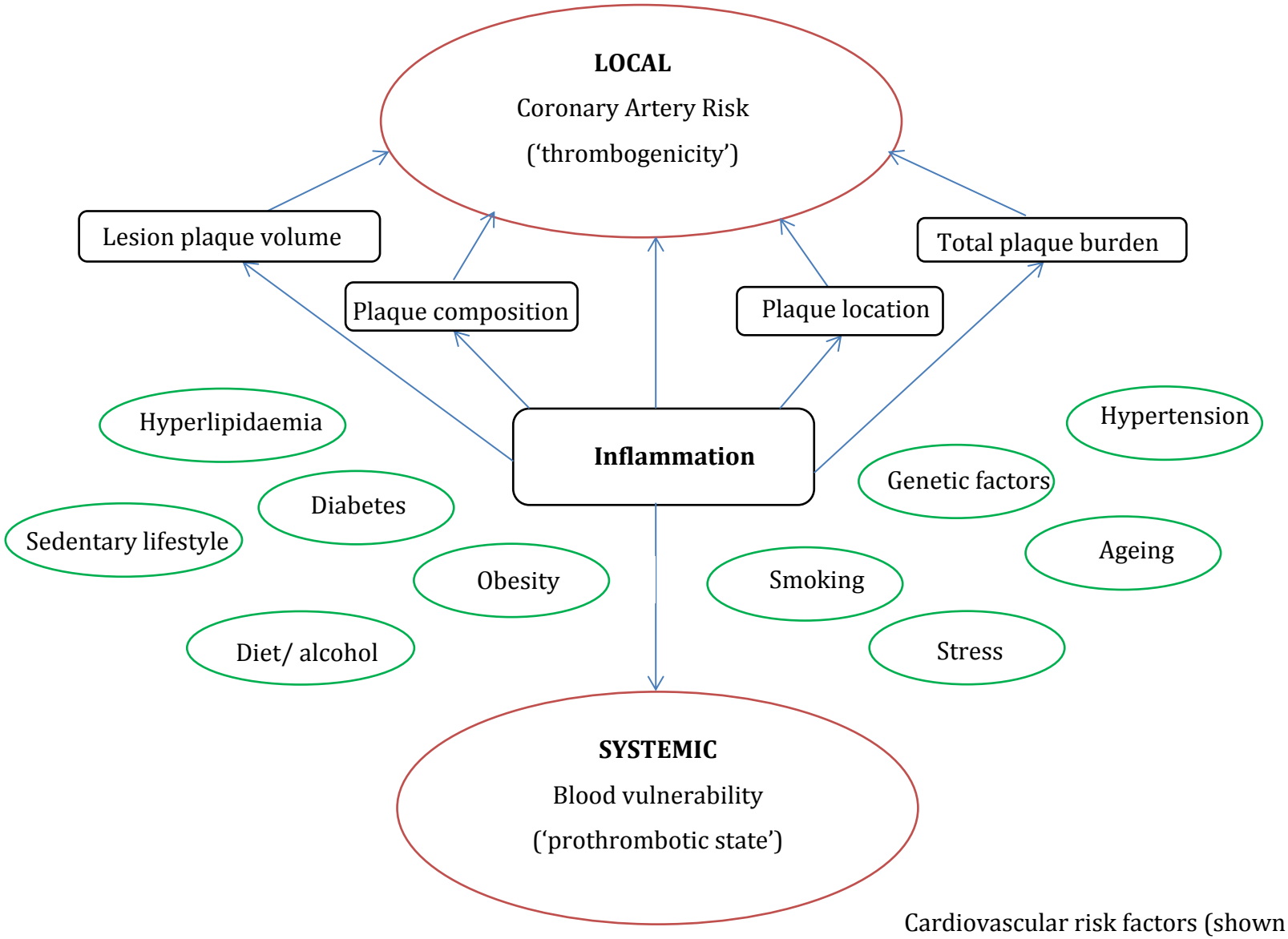
'Traditional' cardiovascular risk factors were first investigated comprehensively through a landmark population-based cohort study, founded in Framingham, Massachusetts in 1949[45]. Hypertension, hypercholesterolaemia, cigarette smoking and overweight/ obesity were clearly established as risk factors for development of atherosclerosis. Other factors including diabetes mellitus, psychosocial stress, physical inactivity, lack of fruit and vegetable consumption and extremes of alcohol intake have since been added to this list supported by evidence from various prominent studies[2,3,46,47]. Estimates of the population attributable risk of AMI for these combined modifiable risk factors range from 70% to 90%[3]. There are various risk prediction scores[48,49] based on combinations of risk factors which are designed to identify people at highest risk from cardiovascular disease as targets for individual interventions. Nonetheless many acute cardiovascular events occur in people with low risk scores. Despite the identification of several promising novel risk factors or markers, such as carotid intima-media thickness, high sensitivity CRP, coronary artery calcium score and genetic risk scores[50], AMI risk is still incompletely explained.

1.4.3 Transition from stable to unstable atherosclerotic disease

Almost all AMIs occur against a background of atherosclerotic coronary artery disease, whether or not this has been diagnosed. A detailed description of the pathogenesis of atherosclerosis, particularly focussing on the potential for interaction with influenza is given in chapter 7. The widespread presence of coronary atherosclerosis at autopsy (eg >80% in US adults with a mean age of 36 years who died of non-natural causes) far exceeds the annual incidence of AMI and sudden cardiac death (estimated in the United States to be between 0.2 and 1%)[51]. This suggests that factors other than the presence of atherosclerosis need to be present for an AMI – characterised by partial of complete epicardial coronary artery occlusion – to occur[51]. Certain features of plaque morphology

combined with the concept of 'vulnerable blood' or a 'prothrombotic state' predispose to AMI. Systemic inflammation is thought to play a key role in development of coronary instability, with or without plaque rupture[52]. This is based on clinical and autopsy reports of widespread coronary and myocardial inflammation in patients with severe unstable angina or infarction[53,54]. Figure 1.1 shows a schematic drawing of the interaction between vascular risk factors and local and systemic conditions necessary for an AMI to occur.

Figure 1.1 Interaction between cardiovascular risk factors and local and systemic systems necessary for AMI
Adapted from Arbab-Zadeh A, et al[51]



Cardiovascular risk factors (shown in green) interact with each other and at multiple points in the pathogenesis of local thrombogenicity and systemic blood vulnerability.

1.4.4 Acute triggers of AMI

As well as studies of traditional cardiovascular risk factors, there is a body of literature focussed on acute triggers of cardiovascular events[55,56]. These may be physical, chemical or psychological factors that represent the final step in the pathophysiological process from chronic atherosclerotic disease to plaque disruption and thrombosis[55]. In vulnerable individuals these factors, which include infection, air pollution, heavy exercise, sexual activity and emotional stress[56], are thought to precipitate acute cardiovascular events through their transient vasoconstrictive and prothrombotic effects[55]. Figure 1.2 (adapted from Mittleman and Mostofsky[55]) is a schematic diagram of some proposed mechanisms linking various acute triggers to AMI in vulnerable individuals. Molecular level inflammatory and haemostatic changes associated with influenza and potential interactions with the final common pathway towards AMI are described further in chapter 7.

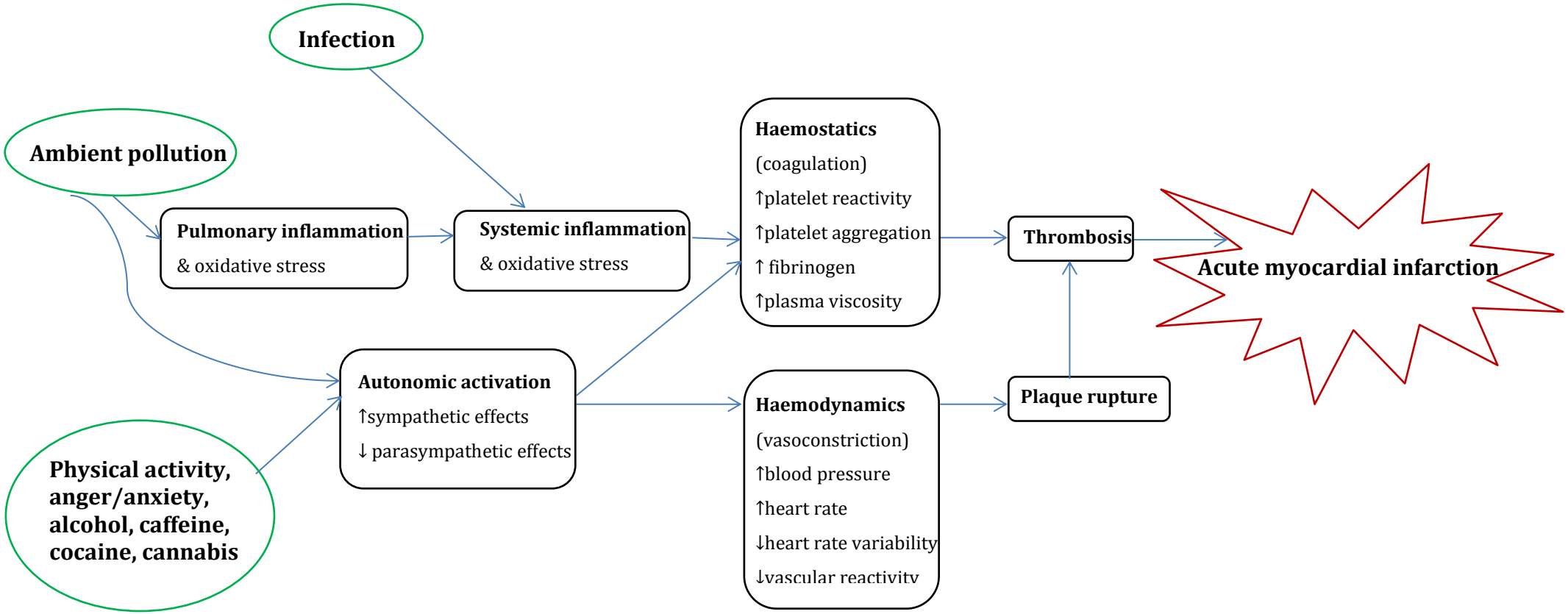


Figure 1.2 Proposed mechanisms through which acute triggers of AMI might act in vulnerable individuals
Adapted from Mittleman and Mostofsky[55]

1.4.5 Definition of AMI

A universal definition of AMI was first developed by an international taskforce in 2000[57] and updated in 2007[58] and 2012[59]. This superseded the previous World Health Organization definition based on symptoms, ECG changes and enzymes to reflect the development of newer sensitive troponin assays for detection of myocardial necrosis[44]. The universal definition of myocardial infarction from 2007 (which was current during the time period of this PhD) is shown is box 1, reproduced from the European Society of Cardiology, American College of Cardiology Foundation, American Heart Association and the World Heart Federation expert consensus document[58].

Criteria for acute myocardial infarction (Universal definition 2007[58], current during this PhD)

The term 'myocardial infarction should be used when there is evidence of myocardial necrosis in a clinical setting consistent with myocardial ischaemia. Under these conditions any one of the following criteria meets the diagnosis for myocardial infarction:

1. Detection of a rise in cardiac biomarkers (preferably troponin) with at least one value above the 99th percentile of the upper reference limit together with evidence of myocardial ischaemia with at least one of the following:
    - Symptoms of ischaemia
    - ECG changes indicative of new ischaemia (new ST-T changes or new left bundle branch block)
    - Development of pathological Q waves in the ECG
    - Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
2. Sudden unexpected cardiac death involving cardiac arrest, often with symptoms suggestive of myocardial ischaemia and accompanied by presumably new ST elevation, or new LBBB and/or evidence of fresh thrombus by coronary angiography and/ or at autopsy, but death occurring before blood samples could be obtained or at a time before appearance of cardiac biomarkers in blood
3. Pathological findings of an acute myocardial infarction

In this definition criteria are also set out for percutaneous coronary intervention-related AMI and coronary artery bypass grafting-associated AMI but these are less relevant to studies included in this PhD so are not described here.

In 2012, the definition was updated again to recognise development of even more sensitive assays for myocardial necrosis especially when such necrosis occurs in the setting of critical illness, cardiac surgery or percutaneous coronary procedures. As changes to the definition were minor and post-dated studies in this thesis, they have not been included in this summary.

1.4.6 Management of AMI

Briefly, the immediate management of AMI depends upon using pharmacological and cardiac catheter-based treatments to restore epicardial and microvascular blood flow, while optimising antithrombotic therapies to suppress recurrent ischaemic events and delivering treatments to mitigate the effect of myocardial necrosis[44]. These will not be discussed further here. Subsequent in-hospital care aims to initiate therapies or lifestyle changes to improve long-term outcome and act as secondary prevention of future events[60]. Key points at which a relationship with influenza might theoretically be relevant for AMI management are prior to first AMI (eg using influenza vaccine for primary prevention), after an AMI (eg using influenza vaccine for secondary prevention) or during an influenza-like illness (eg using antiviral or other medications to lessen symptom severity and reduce cardiovascular risk). These themes will be discussed in chapter 8 (sections on policy implications and future research directions) in light of research findings from this thesis.

1.5 Thesis rationale and aims

Both influenza and AMI are common and contribute to a substantial burden of morbidity and mortality worldwide. AMI remains a key challenge in the clinical management of ischaemic heart disease, especially as it can occur in individuals not previously identified to be at high cardiovascular risk. Improved understanding of the role of inflammatory triggers such as influenza and their contribution to AMI disease burden will both inform strategies for prevention and enhance seasonal and pandemic influenza planning.

1.5.1 Aims and objectives

The overall aim of this thesis is to generate evidence to improve understanding of the relationship between acute respiratory infections, specifically influenza, and acute myocardial infarction and thereby inform policy and practice.

Specific objectives (outlined in more detail in the relevant chapters) are:

1) To investigate and synthesise the evidence from previous studies for a relationship between influenza and AMI or death from cardiovascular disease (chapter 2).
2) To compare population-level associations between seasonal influenza circulation and AMI under different climatic and environmental conditions in two discrete geographical settings (chapter 3).
3) To investigate temporal associations between GP consultations for acute respiratory infections and AMI using a range of linked data sources (chapters 4 and 5).
4) To perform a primary case control study investigating the experience of recent influenza or acute respiratory infection in hospitalised AMI patients and controls during the 2009 influenza A H1N1 pandemic (chapter 6).
5) To examine potential biological mechanisms for an association between influenza and AMI in the literature and through an exploratory study in AMI patients (chapter 7).
6) To discuss research findings, strengths and weaknesses as well as clinical and policy implications (chapter 8).

SUMMARY

- Influenza infection may lead to cardiovascular complications and deaths among vulnerable people
- AMI risk is incompletely explained by traditional vascular risk factors
- The contribution of inflammatory triggers such as influenza to AMI disease burden is not well understood
- This thesis will investigate the relationship between acute respiratory infections, specifically influenza, and AMI through review of existing literature and by presenting original research to generate evidence to inform influenza prevention, treatment and planning

2. Systematic literature review of the relationship between influenza and acute myocardial infarction or death from cardiovascular disease

2.1 Description of chapter contents

In this chapter I describe a systematic literature review of the evidence that influenza (including influenza-like illness and acute respiratory infection) might trigger AMI or cardiovascular death. I also examine the effectiveness of influenza vaccine at protecting against cardiac events through performing a meta-analysis of data from randomised controlled trials. Systematic searches are done of electronic databases and hand-searches of reference lists from relevant articles for original research articles matching inclusion criteria in any language. Results from included studies are grouped by study design and by influenza classification. The quality of evidence is assessed by first considering flaws common to each study design and then by examining studies individually. Conclusions are drawn from available evidence and outstanding questions are highlighted. Updated evidence from recent searches is provided in an appendix to this chapter.

2.2 Study rationale

Although some cardiac complications of influenza infection such as myocarditis are well-recognised, the nature of any relationship between influenza and AMI is less clear. Before embarking on original research studies, a systematic review was needed to identify whether existing studies provided evidence to support the hypothesis that AMI can be triggered by influenza infection and to identify gaps in the literature.

2.3 Aims and objectives

Aim: to perform a systematic review of the evidence for a relationship between influenza and AMI or death from cardiovascular disease.

Objectives:

1) To design and perform systematic searches to identify original studies examining the link between influenza (including laboratory-confirmed influenza, influenza-like illness and acute respiratory infection in the absence of other aetiological information) or influenza vaccine and AMI or death from cardiovascular disease.
2) To extract data from studies meeting inclusion criteria using a purpose-designed data extraction tool.
3) To synthesise findings by study design and influenza classification.
4) To perform a meta-analysis of data from randomised controlled trials.
5) To critically appraise quality of evidence.

2.4 Methods

2.4.1 Search strategy

Searches were originally carried out using Pubmed (up to Feb 2009) and Embase (1980 – Feb 2009) to describe the literature at the beginning of this PhD. I used the following MeSH search terms: "influenza human" OR "influenza vaccines" OR "viruses"[Majr] OR "respiratory tract infections"[Majr] AND "myocardial infarction". The search was repeated with the MeSH terms "influenza human" OR "influenza vaccines" AND "cardiovascular diseases"[Majr]. Keyword searches were performed in both databases using all possible combinations of terms from the following lists: 1) "influenza", "flu", "vaccine" and "respiratory infection" and 2) "myocardial infarction", "cardiovascular", "coronary", "atherosclerosis" and "atherogenesis". Bibliographies of relevant articles were searched for additional references. Findings were updated in October 2012 with additional results presented in an appendix to this chapter (appendix 2a, p65).

2.4.2 Inclusion and exclusion criteria

I included original studies with the outcomes 'myocardial infarction' or 'death from cardiovascular disease' (or death from a more specific cause such as coronary heart disease, or ischaemic heart disease). I classed relevant exposures

as a) influenza, b) influenza-like illness, c) acute respiratory infection (where this was not subdivided into causes), and d) influenza vaccination. I included ecological, case control, cohort, case only studies (eg self-controlled case series or case crossover) and randomised controlled trials but excluded case reports, review articles, editorials, clinical guidelines and information articles for patients. Studies that examined intermediate outcomes such as inflammatory markers, cardiac enzyme levels or ECG changes (outside the context of AMI) were rejected. Studies where methods were insufficiently described were also rejected. All case control and cohort studies were required to have a comparison group to enable relative measures of the effect of influenza to be generated. Similarly, all case only studies needed to have a comparison time period. I considered studies from any time period, any country and in any language for inclusion. For potentially relevant studies published in languages other than English, French and Italian, I advertised for PhD students to translate articles for a small payment. Translators assisted with articles published in Russian, German and Polish.

2.4.3 Data extraction and synthesis

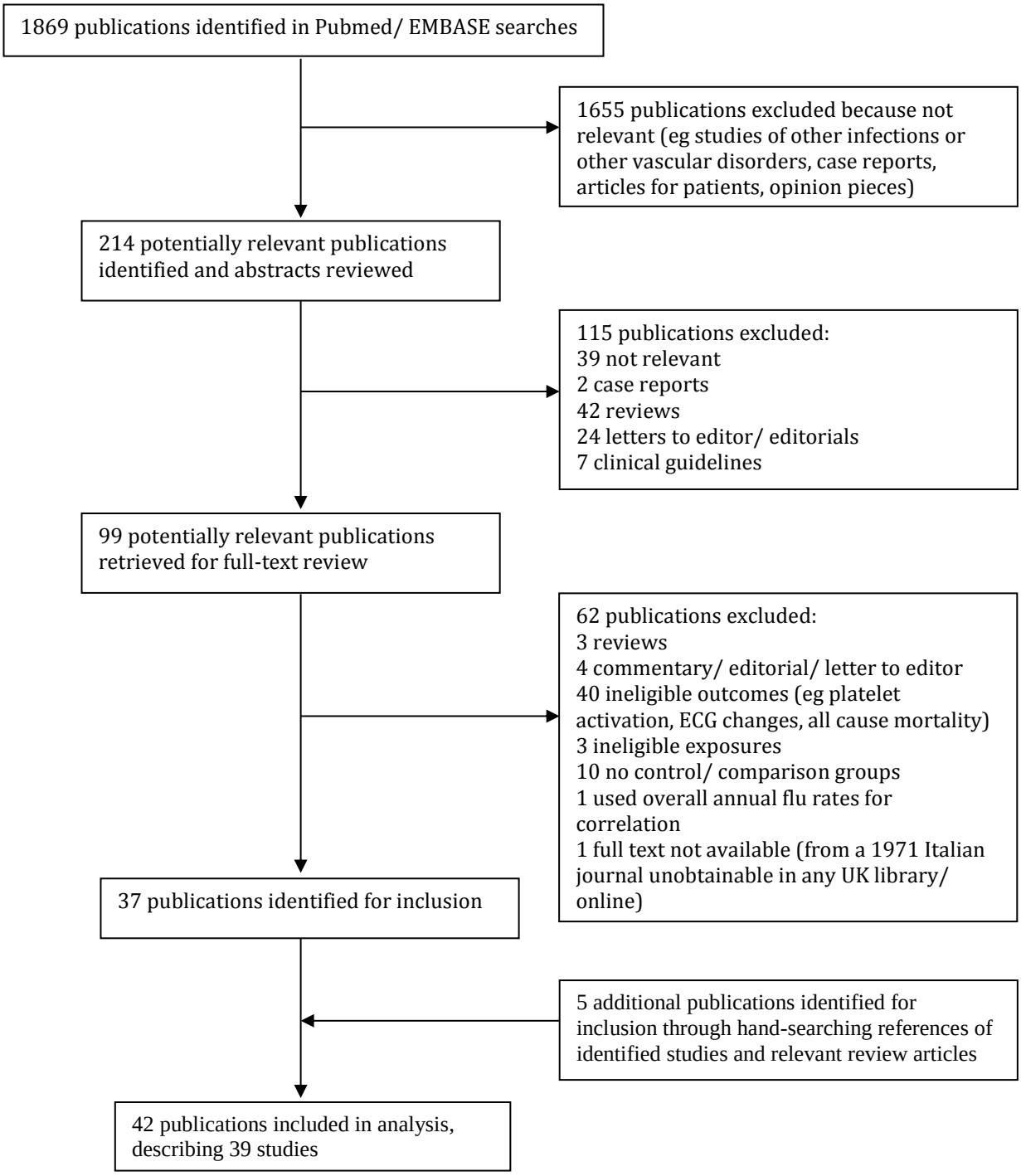
I collected data on study populations, settings, exposure definitions, outcome definitions, outcome measures, effect sizes, confidence intervals, and results of significance testing, using a data extraction sheet that I had designed. Studies were categorised by study design and by definitions of influenza (confirmed influenza, influenza-like illness, and acute respiratory infection). For observational studies no formal meta-analysis was done, as factors such as study design, exposure definitions, likelihood of bias and the extent to which authors controlled for confounding were too heterogeneous across studies. Where possible, though, I compared effect sizes within categories of study type. For randomised controlled trials of the effect of influenza vaccination on cardiac outcomes, I performed a meta-analysis using the user written 'metan' suite of commands[61] in Stata version 10, which produced summary effects using both fixed- and random-effects models. The likelihood of bias for individual studies was assessed against criteria defined in the STROBE statement[62], tailored to the study question. General quality issues were reported by study type to reflect flaws common to certain study designs.

2.5 Results

2.5.1 Search results

The search yielded 1,869 articles, of which 99 were deemed relevant and 37 fulfilled inclusion criteria (figure 2.1). An additional five articles were identified by scanning reference lists of included studies and relevant reviews. My analysis included 42 papers published between 1932 and 2008. Of these, 17 described ecological studies, 15 reported case control studies, three were prospective cohort studies, two were case only studies (incorporating one self-controlled case series study and one case crossover), and five papers described randomised controlled trials (including four papers describing outcomes of the same trial at different time points). 16 papers included the outcome 'myocardial infarction' alone, six incorporated AMI as part of a composite outcome and 20 used the outcome 'cardiovascular death' (with variants including 'death from coronary heart disease', 'death from ischaemic heart disease', 'death from organic heart disease', and 'death from arteriosclerotic heart disease').

Figure 2.1 Study flow chart



2.5.2 Overview of study designs

Various study designs have been used to study the relationship between influenza and cardiovascular disease. Analytical ecological studies attempting to correlate population measures of influenza exposure with population level data on cardiovascular mortality typically generated correlation coefficients. Case control

studies, usually comprising AMI patients and controls sampled from a similar underlying population, yielded estimates of the relative effect of influenza on AMI. Case only studies, such as self-controlled case series or case crossover studies, compared the relative incidence of AMI occurring in different time periods after influenza infection using cases as their own controls. This review did not include any cohort studies used directly to study the relationship between influenza and AMI. Several prospective cohort studies were included, however, that investigated any relative protective effect of influenza vaccination against AMI in individuals who had received influenza vaccine compared to an unvaccinated comparison group. Similarly, randomised controlled trials enabled the occurrence of AMI or cardiovascular death to be compared between groups randomised to receive either influenza vaccine or no vaccine/placebo. Characteristics of ecological studies, individual observational studies and randomised controlled trials are described in tables 2.1, 2.2 and 2.5 respectively.

Authors	Study location	Study years	Exposure(s)	Definition of exposure(s)	Outcome(s)	Definition of outcome(s)
Collins (1932)[29]	USA	1917 - 1929	Influenza epidemics	Weekly mortality rates from influenza & pneumonia	Organic heart disease mortality	Weekly excess mortality rates from organic heart disease
Azambuja & Duncan (2002)[63]	USA	1918 and 1920 - 1985	Influenza circulation	Influenza mortality rates in 1918	Coronary heart disease mortality	Annual mortality rates from coronary heart disease in 1920 - 1985
Bainton, Jones & Hole (1978)[64]	London, UK	1953 - 1973	Influenza circulation	a) Weekly mortality rates from influenza, pneumonia, bronchitis & all causes b) Weekly ILI incidence seen in General Practice c) Laboratory influenza surveillance data d) New sickness benefits	Arteriosclerotic and ischemic heart disease mortality	Weekly mortality rates from arteriosclerotic or ischaemic heart disease
Eickhoff, Sherman & Serfling (1961)[65]	USA	1957 – 1960	Influenza epidemics	Weekly mortality rates from influenza & pneumonia	Cardiovascular- renal disease mortality (with subcategory arteriosclerotic heart disease mortality)	Weekly excess mortality rates from cardiovascular & renal disease
Housworth & Langmuir (1974)[66]	USA	1957 – 1966	Influenza circulation	Influenza surveillance data – surveillance method unclear.	Heart, circulatory & nervous system mortality (with subcategory arteriosclerotic heart disease mortality)	Monthly excess mortality rates from heart, circulatory & nervous system disease
Duda et al (1974)[67]	Romania	1956 - 1971	Influenza circulation	Monthly and annual influenza morbidity – surveillance method unclear	Cardiovascular disease mortality	Monthly and annual mortality rates from cardiovascular disease
Reichert et al (2004)[68]	USA	1959 - 1999	Influenza circulation	a) Monthly mortality rates from influenza b) Laboratory influenza surveillance data	Ischaemic heart disease mortality	Monthly excess mortality rates from ischaemic heart disease

Scragg R (1985)[69]	Australia	1967 - 1977	Influenza epidemics	Monthly and annual mortality rates from influenza	Ischaemic heart disease mortality	Monthly and annual excess mortality rates from ischaemic heart disease
Cooper et al (1980)[70]	USA	1968 - 1976	Influenza circulation	Monthly and annual mortality rates from influenza	Coronary heart disease mortality	Monthly and annual mortality rates from coronary heart disease
Tillett, Smith & Gooch (1983)[71]	UK	1968 - 1978	Influenza circulation	a) Weekly mortality rates from respiratory disease b) Weekly ILI incidence seen in General Practice	Circulatory disease mortality (with subcategory ischaemic heart disease mortality)	Weekly excess mortality rates from circulatory disease
Alling, Blackwelder & Stuart-Harris (1981)[72]	USA	1968 - 1976	Influenza circulation	a) Monthly mortality rates from influenza b) Laboratory influenza surveillance data	Cardiovascular disease mortality	Monthly and annual excess mortality rates from cardiovascular disease
Marshall, Scragg & Bourke (1988)[73]	New Zealand	1970 - 1983	Influenza circulation	Monthly mortality rates from respiratory disease	Coronary heart disease mortality	Monthly mortality rates from coronary heart disease
Dvorakova & Poledne (2004)[74]	Czech Republic	1973 - 1997	Influenza circulation	Influenza surveillance data – surveillance method unclear	AMI incidence	Hospitalisations for AMI identified from disease-specific register in regional hospital of Central Bohemia. AMI definition unclear.
Mackenbach, Kunst & Looman (1992)[75]	Holland	1979 - 1987	Influenza circulation	National mortality rates from respiratory disease	Cardiovascular disease mortality (with subcategory ischaemic heart disease mortality)	Daily mortality rates from cardiovascular disease
Madjid et al	Russia	1993 - 2000	Respiratory	a)Weekly incidence of acute	Coronary heart disease	Weekly incidence of

(2007)[76]			disease and influenza epidemics	respiratory disease b) Laboratory influenza surveillance data	mortality	autopsy-confirmed AMI or chronic IHD deaths
Fleming, Cross & Pannell (2005)[30]	UK	1994 - 2000	Influenza circulation	a) Weekly ILI incidence seen in General Practice b) Laboratory influenza surveillance data	Circulatory disease mortality (with subcategory ischaemic heart disease mortality)	Weekly mortality rates from circulatory disease
Saltykova et al (2008)[77]	Russia	1999 - 2005	Influenza circulation	National mortality rates from influenza	Cardiovascular disease mortality (with subcategory AMI mortality)	National mortality rates from cardiovascular disease

Table 2.1 Characteristics of ecological studies, n=17

Authors	Study location	Study design	Study population	Exposure(s)	Definition of exposure(s)	Outcome(s)	Definition of outcome(s)	Sample size
Spodick, Flessas & Johnson (1984)[78]	USA	Matched case control	Cases - AMI patients Controls - matched for age, sex & date of hospital admission	Respiratory infection	Clinical history of respiratory symptoms	AMI	AMI definition not stated	150 cases 150 controls
Penttinen & Valonen (1996)[79]	Finland	Nested case control	Cases - AMI patients Controls - from within a community cohort of farmers, matched on dob, SES and smoking status	Respiratory infection	GP presentation with respiratory symptoms	AMI	AMI diagnosis obtained from hospital discharge register, death certificates, and medical records	83 cases 249 controls
Meier et al (1998)[80]	UK	Matched case control	Cases - AMI patients in GPRD Controls - patients without cardiac risk factors matched on age, sex & GP	Respiratory infection	GP presentation with systemic respiratory infection	AMI	AMI Read codes in primary care records	1922 cases 7649 controls
Meyers et al (2004)[81]	USA	Case control	Cases - hospitalised AMI patients Controls - hospitalised fracture patients	a) Respiratory infection b) Influenza vaccine	a) Clinical symptoms b) Self-reported vaccination status	AMI	At least two of: - Typical chest pain - ECG changes - Rise in troponin/ CK-MB/ myoglobin - Occluded coronary artery on angiography	335 cases 199 controls
Clayton et al (2005)[82]	UK	Matched case control	Cases - hospitalised AMI patients Controls - from community, matched on age, sex & deprivation	Respiratory infection	Clinical history of respiratory symptoms	AMI	Clinical diagnosis of AMI (made in a coronary care unit)	119 cases 214 controls
Clayton et al (2008)[83]	UK	Matched case control	Cases - Patients with a first AMI in the 'IMS Disease Analyzer Mediplus Primary Care	Respiratory infection	GP presentation with systemic respiratory infection (using	AMI	AMI Read codes in primary care records	11, 155 cases 11, 155 controls

			Database'. Controls - from same database matched on age, sex, GP and time		primary care records)			
Smeeth et al (2004)[84]	UK	Self-controlled case series	Cases - GPRD patients with a history of AMI and respiratory infection or flu vaccination	a) Respiratory infection b) Influenza vaccination	a) GP presentation with systemic respiratory infection b) Flu vaccination status using primary care records	AMI	AMI Read codes in primary care records	20, 486 cases
Zheng et al (1998)[85]	USA	Case crossover	Cases - identified from a research database of AMI patients	Influenza-like illness	Clinical symptoms (fever and sore throat)	AMI	AMI definition not stated	2,264 cases
Pesonen et al (2008)[86]	Sweden	Matched case control	Cases - AMI patients Controls - from community, matched on age, sex, parish, residence, season & flu circulation	Influenza-like illness	Clinical symptoms	AMI	At least two of: - typical ECG changes - chest pain lasting >20 minutes - raised CK-MB	110 cases 323 controls
Nicholls & Thomas (1977)[87]	UK	Case control	Cases - AMI patients in coronary care unit Controls - patients admitted to coronary care unit without AMI	a) Influenza-like illness b) Influenza	a) Clinical symptoms b) Antibodies to influenza A infection (complement fixation tests on paired sera)	AMI	According to WHO definition 1959	38 cases 21 controls
Ponka et al (1981)[88]	Finland	Case control	Cases - AMI patients Controls - acute admissions for non-cardiac reason	a) Influenza-like illness b) Influenza	a) Clinical symptoms b) Antibodies to influenza A in	AMI	Clinical history, ECG changes, and elevated CK-MB levels	49 cases 37 controls

					serum			
Mattila (1989)[89]	Finland	Case control	Cases - AMI patients Controls - group 1: random patients; group 2: patients with chronic CHD	a) Influenza-like illness b) Influenza	a) Clinical symptoms b) IgM and IgG antibodies to influenza in serum	AMI	Ischaemic symptoms, ECG changes, and elevated CK-MB	40 cases 41 controls (group 1) 30 controls (group 2)
Porter & Porter (1999)[90]	USA	Case control	Cases - patients dying of cancer Controls - patients dying of AMI (autopsy-confirmed)	Influenza	Influenza virus antigens in lung tissue	Cancer death	Diagnosed by autopsy	118 cases 20 controls
Guan et al (2008)[91]	China	Case control	Cases - hospitalised AMI patients Controls - patients attending occupational health	Influenza	IgG antibodies to influenza in serum	AMI	Ischaemic symptoms, ECG changes, and raised cardiac enzymes	99 cases 110 controls
Naghavi et al (2000)[92]	USA	Case control	Cases - hospitalised AMI patients Controls - CHD patients admitted with no AMI	Influenza vaccination	Influenza vaccination status	AMI	At least two of: - ECG changes - enzyme changes - typical chest pain	109 cases 109 controls
Siscovick et al (2000)[93]	USA	Case control	Cases - patients dying of primary cardiac arrest Controls - randomly selected from community	Influenza vaccination	Self-reported influenza vaccination status	Primary cardiac arrest	Clinical diagnosis	342 cases 549 controls
Heffelfinger et al (2006)[94]	USA	Nested matched case control	Cases - AMI patients Controls - from a cohort of patients attending a group health co-operative matched for age, sex, year and BP	Influenza vaccination	Vaccination status obtained from medical records	AMI	From medical records. Confirmed by symptoms, cardiac enzymes, ECG findings, provider notes & discharge summaries	750 cases 1735 controls

Jackson et al (2002)[95]	USA	Cohort	Cohort - patients with a first AMI	Influenza vaccination	From medical records and administrative data systems	a) Nonfatal AMI + CVD death b) Fatal and nonfatal AMI	AMI = chest pain, ECG changes and cardiac enzyme rise.	1,378 patients (127 events from Aug '92 - Dec 96. Median follow up = 2.3 years)
Armstrong et al (2004)[96]	UK	Cohort	Cohort - elderly people registered with general practices	Influenza vaccination	Medical record of vaccination	Mortality from CVD	CVD mortality rates from Office for National Statistics	24,535 patients (2,193 events from Jan '96 - Aug '00)
Wang et al (2007)[97]	Taiwan	Cohort	Community cohort of elderly people	Influenza vaccination	Vaccination status from administrative records	Mortality from CVD	Mortality data from death certificates	102,698 patients (484 events from Jan 1st '01 - Oct 31st 01)

Table 2.2 Characteristics of individual observational studies, n=20

2.5.3 Ecological studies

2.5.3.i Results of ecological studies

17 ecological studies examined the association between timing of influenza circulation and either mortality from cardiovascular disease (CVD)[29,30,63–73,75–77] or incidence of AMI[74] using regression modelling (seven studies) or comparing numbers or rates of deaths in influenza and non-influenza circulation periods (ten studies). Studies were carried out in various temperate climates worldwide including the USA(7), UK(3), Russia(2), Australia(1), Czech Republic(1), Holland(1), New Zealand(1), and Romania(1). All 17 reported an increase in CVD mortality or AMI incidence during time periods when influenza was circulating. Six studies generated correlation coefficients for associations between weekly or monthly rates of influenza circulation and death rates from CVD using methods such as Spearman's rank correlation test and time series cross correlation analysis. These ranged from 0·61 - 0·77 in five studies[63,67,72,73,77]. The final study reported age-specific correlation coefficients of 0·77 (for those aged 45-64), 0·87 (for those aged 65-75) and 0·98 (for the over 75s)[30]. Overall these suggest a medium to strong correlation. Another two studies found that significantly more deaths from ischaemic heart disease occurred during influenza epidemic weeks compared to non-epidemic weeks[64,76]. Two studies reported correlation coefficients between influenza circulation and death from AMI of 0·38 and 0·5[76,77].

Some ecological studies estimated the overall excess mortality secondary to influenza (or influenza-like illness), for example by using national surveillance systems to calculate numbers of deaths in periods when influenza was circulating that exceeded a baseline number of expected deaths in time periods when influenza activity was absent. Overall excess influenza mortality was then divided into causes. Nine studies reported a percentage of excess influenza deaths that were due to CVD[29,30,65,66,68,69,71,72,75]; these ranged from 18% (in the 1918 pandemic in the USA)[29] to 57% (in 1968/69 in the USA)[68]. Generally the proportion of excess influenza deaths due to CVD averaged around 35-50% but changing definitions of CVD made figures difficult to compare across studies.

2.5.3.ii Limitations of ecological studies

A major limitation to such studies is ecological bias or failure of ecological associations to reflect biological effects at an individual patient level. The assumption that those individuals dying of cardiovascular disease have been exposed to influenza may be an example of this ecological fallacy. Many ecological studies were designed to investigate overall influenza mortality rather than cardiovascular mortality specifically; several studies were excluded for using overly broad definitions of CVD death eg 'respiratory and circulatory deaths'[98–100] which would have introduced bias. Another substantial problem with ecological studies is the difficulty controlling for potential population level confounding factors such as low temperature and humidity. In one study daily minimum temperature averaged across 4-week periods was included as a covariate in multivariable regression models[71]; in another study, time periods when influenza was and was not circulating were matched for temperature and numbers of excess CVD deaths compared[64]; in both studies the effect remained evident after controlling for temperature. The remaining studies, however, did not control appropriately for temperature. In analysing correlations between influenza circulation and CVD mortality, it may also be necessary to allow a time lag of eg 1-2 weeks during which an effect can take place. Nevertheless few studies included a lag period, which itself may be difficult to determine accurately: surveillance data may also lag behind true community incidence of infection[101]. Despite this, ecological studies are useful for generating hypotheses to examine in further detail using individual level data, as well as for estimating public health burden (using measures such as excess mortality).

2.5.4 Individual observational studies – case only, case control and cohort

2.5.4.i Acute respiratory infections

Seven observational studies (six case control studies[78–83] and one self-controlled case series study[84]) examined the experience of recent acute respiratory infection in AMI patients by self-reported symptoms or from GP records. Five reported statistically significant associations between occurrence of recent respiratory symptoms and AMI, with effect estimates ranging from 2·1 (95% CI 1·4 - 3·2)[83] to 4·95 (95% CI 4·4 - 5·5)[84]. One study showed no effect of recent respiratory

infection (OR 1·0 (95% CI 0·5 - 1·9), p=0·98) but demonstrated a significantly greater chance of recent fever in cases compared to controls (OR 5·9 (95% CI 2·0 - 16·8), p<0·0001)[82]. The final study suggested that two or more GP attendances with upper respiratory tract infection were associated with AMI, but this association did not hold true when comparing one GP visit with no visits (OR 1·4 (95% CI 0·8 - 2·3), p=0·19)[79]. Three of these studies also described a higher risk of AMI in the days immediately following acute respiratory infection[80,83,84]. The largest of these - a self-controlled case series study of over 20,000 UK patients with a first AMI in the General Practice Research Database - reported an incidence ratio of 4·95 for AMI occurring in the first 1-3 days after acute respiratory infection, which fell to 3·2 on days 4-7, 2·8 on days 8-14 and 1·4 on days 15-28[84]. A similar gradient of effect sizes was reported for two case control studies performed in primary care databases, with OR 2·1 (95% CI 1·4 - 3·2) on days 1-7 in one study[83] and OR 3·6 (95% CI 2·2 - 5·7) on days 1-5 in the other[80].

2.5.4.ii Influenza-like illness

A further five studies (four case control studies[86–89] and one case crossover study[85]) used the more specific exposure of influenza-like illness (ILI), defined using syndromes of clinical symptoms such as fever plus sore throat (though definitions varied across studies). Of the case control studies, two reported a statistically significant positive association between recent ILI and AMI, with odds ratios of 3·8 (95% CI 1·4 - 10·8), p=0·011[86] and 3·0 (95% CI 1·1 - 8·2), p=0·03[89]. The other two case control studies showed a slight positive but non-significant association, with odds ratios of 1·7 (95% CI 0·5 - 5·6), p=0·41 and 1·2 (95% CI 0·3 - 4·4), p=0·84[87,88]. In a case crossover study of 2,264 patients with AMI, 19% reported a recent ILI[85]. In these patients, the relative probability that an AMI occurred on the first day after ILI onset compared to seven days afterwards was 2·4 (95% CI 1·7 - 3·4).

2.5.4.iii Influenza (laboratory-confirmed)

Four case control studies examined influenza exposure by testing either single serum samples for IgG antibodies using ELISA[91] or by using complement fixation assays to detect influenza antibodies in paired acute and convalescent samples[87–89]. Only the first of these reported a highly significant odds ratio, for IgG

antibodies to influenza A (OR 7·5 (95% CI 1·3 - 47·0), p=0·023) and influenza B (OR 27·3 (95% CI 6·6 - 113·8), p<0·001) in AMI cases compared to controls[91]. Two other studies reported non-significant associations (OR 0·9 (95% CI 0·2 - 3·1), p=0·81)[87] and OR 0·5 (95% CI 0·1 - 2·6), p=0·44)[88]; in the fourth study it was not possible to calculate an odds ratio as no influenza antibodies were detected in either group[89]. A further case control study examined the prevalence of influenza virus antigen in the lungs of deceased patients on autopsy[90]. Comparing patients who died of AMI to those dying of cancer revealed no significant difference - OR 1·0 (95% CI 0·1 - 8·6), p=0·99. Results of individual observational studies are shown in table 2.3.

Authors (year)	Study design	Sample size	Exposure (definition)	Odds ratio (95% CI)	P value
Zheng (1998)[85]	Case crossover	2, 264 cases	Influenza-like illness (symptoms)	2·4 (1·7-3·4)	-
Pesonen (2008)[86]	Case control	110 cases 323 controls	Influenza-like illness (symptoms)	3·8 (1·4-10·8) for 2-3 symptoms v 0-1 symptoms	0·011
Nicholls (1977)[87]	Case control	38 cases 21 controls	1) Influenza-like illness (symptoms)	1·7 (0·5-5·6)*	0·41*
			2) Influenza (antibodies in paired sera)	0·9 (0·2-3·1)*	0·81*
Ponka (1981)[88]	Case control	49 cases 37 controls	1) Influenza-like illness (symptoms)	1·2 (0·3-4·4)*	0·84*
			2) Influenza (antibodies in paired sera)	0·5 (0·1-2·6)*	0·44*
Mattila (1998)[89]	Case control	40 cases 71 controls	1) Influenza-like illness (symptoms)	3·0 (1·1-8·2)*	0·03*
			2) Influenza (antibodies in paired sera)	No influenza antibodies detected in either group	-
Porter (1999)[90]	Case control	20 cases 118 controls	Influenza (viral antigen in lung tissue)	1·0 (0·1-8·6)*	0·99*
Guan (2008)[91]	Case control	99 cases 110 controls	1) Influenza A (IgG in single serum sample)	7·5 (1·3-43·0)	0·023
			2) Influenza B (IgG in single serum sample)	27·3 (6·6-113·8)	<0·001
Spodick (1984)[78]	Case control	150 cases 150 controls	Respiratory infection (symptoms)	2·19 (no CI given)	<0·02

Penttinen(1996)[79]	Case control	83 cases 249 controls	Respiratory infection (No. of GP visits)	3·2 (1·2-8·5) for 4 v 3 visits 1·4 (0·8-2·3) for 1 v 0 visits	0·01 0·19
Meier (1998)[80]	Case control	1,922 cases 7,649 controls	Respiratory infection (GP visit)	3·6 (2·2-5·7) on days 1-5 2.3 (1·3-4·2) on days 6-10 1·8 (1·0-3·3) on days 11-15 1·0 (0·7-1·6) on days 16-30	<0·01 (test for trend)
Meyers (2004)[81]	Case control	335 cases 199 controls	Respiratory infection (symptoms)	2·4 (1·1-5·4)*	0·03*
Clayton (2005)[82]	Case control	119 cases 214 controls	Respiratory infection (symptoms)	1·0 (0·5-1·9)	0·98
Clayton (2008)[83]	Case control	11,155 cases 11,155 controls	Respiratory infection (GP visit)	2·1 (1·4-3·2) on days 1-7 1·9 (1·4-2·6) on days 8-28 1·2 (0·9-1·5) on days 29-91	<0·001 (test for trend)
Smeeth (2004)[84]	Self-controlled case series	20,486 cases	Respiratory infection (GP visit)	#4·95 (4·4-5·5) on days 1-3 3·2 (2·8-3·6) on days 4-7 2·8 (2·5-3·1) on days 8-14 1·4 (1·3-1·5) on days 15-28	-

Table 2.3 Observational studies of association between either presumed influenza infection or non-specified respiratory infection and AMI
* Calculated from crude figures given. #Incidence ratios rather than odds ratios reported

2.5.4.iv Limitations of case control studies

Eight of the 15 included case control studies were judged to be prone to selection bias because of the use of hospital-based control groups that may not have been representative of the population from which cases arose. Control groups were recruited from people admitted or attending for other reasons eg fractures (one study)[81], other cardiac conditions (three studies)[87,89,92], occupational health checks (one study)[91], cancer (one study)[90], and acute non-specified admissions (two studies)[78,88]. It is possible that the reason for hospitalisation among some of these controls was triggered or affected by ILI, which is likely to have biased effect estimates towards the null. The remaining seven case control studies were either nested within cohort studies (two studies)[79,94] or selected controls from the community (five studies)[80,82,83,86,93], so were judged to have been less prone to selection bias.

Recall bias was also potentially a problem in the six case control studies that relied on self-reported vaccination status or self-reported symptoms to diagnose recent acute respiratory infection clinically[78,81,82,86,92,93]. This was less of an issue for four studies that verified this information using general practice records[79,80,83,94] and for the five studies that also used laboratory confirmation of influenza infection[87–91]. Though serological definitions of influenza exposure improve specificity, it can be difficult to estimate the timing of infection basing on detection of antibodies, which also rise in response to vaccination. In three of the four antibody studies, paired acute and convalescent sera were tested to try to establish the timing of antibody rises, though authors did not control for influenza vaccination status[87–89]. The study using a single assay to detect influenza IgG, though unable to assess timing of any antibody rise, was conducted in a Chinese population with an extremely low prevalence of influenza vaccination (two cases and one control reported vaccinations)[91]. Overall six of the 15 case control studies failed to control adequately for potential confounders such as smoking status and influenza vaccination[78,86–90]. Although in general, case control studies are more efficient and therefore require fewer participants than cohort studies, nearly two thirds of included studies were clearly underpowered to detect an effect of influenza on AMI risk.

2.5.4.v Limitations of case only studies

Two case only studies were included in which each individual acted as their own control. As well as improving statistical efficiency, a major advantage of this type of study is the elimination of the effect of fixed confounders such as socio-economic status and health-seeking behaviour. Both studies relied upon medical records or database data, however, so there is likely to have been missing information on other time-varying confounders such as smoking status. Though recall bias may have affected the case crossover study which relied on recall of respiratory symptoms[85], this is unlikely to have varied systematically within the group of AMI patients. The other study used GP episodes to diagnose acute respiratory infections[84]. Although this method substantially underestimates total numbers of patients with acute respiratory infection or influenza-like illness (as most do not present to the GP), it would tend to underestimate any triggering effect of infections on AMI rather than to provide false evidence of effect.

2.5.5 Observational and intervention studies of influenza vaccine

2.5.5.i Observational studies

Eight observational studies - four case control[81,92–94], three cohort[95–97] and one self-controlled case series[84] - compared the incidence of AMI or cardiovascular events in those vaccinated against influenza with those who had not received vaccination. Results were mixed. Three studies showed a protective effect, one showed a slight protective effect that was not statistically significant, and four showed no evidence of effect (table 2.4). These null studies included a small cohort study that was underpowered to detect a protective effect of vaccine[95] and a self-controlled case series study[84], designed to examine whether AMI was triggered by influenza vaccination. This study focussed only on short-term effects, comparing the relative incidence of AMI in the period immediately following influenza vaccination with other time periods for the same individual. As the effect of influenza vaccination may last for several years, it was therefore unlikely to have demonstrated a protective effect (because there would be no true baseline time periods for an individual).

Authors (year)	Study design	Sample size	Outcome	Odds or hazard ratio (95% CI)	P value
Siscovick (2000)[93]	Case control	342 cases 549 controls	Primary cardiac arrest	OR 0·51 (0·33- 0·79)	-
Naghavi (2000)[92]	Case control	109 cases 109 controls	Recurrent AMI	OR 0·33 (0·13-0·82)	0·017
Meyers (2004)[81]	Case control	335 cases 199 controls	AMI	OR 0·90 (0·60-1·35)	0·59
Heffelfinger (2006)[94]	Case control	750 cases 1,735 controls	AMI	OR 0·97 (0·75-1·27)	0·95
Jackson (2002)[95]	Cohort	1,378 subjects (127 events)	1) AMI or CVD death 2) AMI	HR 1·18 (0·79-1·75) HR 1·23 (0·81-1·87)	- -
Armstrong (2004)[96]	Cohort	24,535 subjects (2,193 events)	CVD death	HR 0·87 (0·73-1·02)	0·09
Wang (2007)[97]	Cohort	102,698 subjects (484 events)	'Heart disease' death	HR 0·78 (0·64-0·96)	<0·05
Smeeth (2004)[84]	Self-controlled case series	20,486 cases	AMI	IR 0·8 (0·6-0·9) on days 1-3* IR 0·7 (0·8-1·0) on days 8-14 IR 1·0 (1·0-1·1) on days 15-28	-

Table 2.4 Case control, case only and cohort studies showing the association between influenza vaccination and CVD death or AMI

* Measured incidence ratio (IR) for AMI occurring in time periods immediately following vaccination compared to other time periods in vaccinated individuals.

2.5.5.ii Intervention studies

Two intervention studies[102,103] that were recently included in a Cochrane systematic review[104], used a randomised controlled trial design to examine whether influenza vaccination protected against AMI and CVD death as well as a range of composite cardiovascular outcomes. The FLUVACS study[105] randomised 301 patients (200 post AMI and 101 presenting for planned angioplasty/stent with no history of unstable angina, AMI, coronary artery bypass graft (CABG) or angioplasty) to either influenza vaccine or control groups. Sequential follow up at 6 months, one and two years showed a significantly reduced risk of cardiovascular death in the intervention group, which diminished over time (HR 0·25 (95% CI 0·07 - 0·86), p=0·01 at 6 months[106]; HR 0·34 (95% CI 0·17 - 0·71), p=0·002 at 1 year[105]; HR 0·33 (95% CI 0·07 - 1·59), p=0·14 at 2 years)[107]. Data on subsequent AMI, collected as part of a composite endpoint, showed that there was no effect of vaccine on AMI risk at one year, with equal numbers of events in vaccine and control groups (HR 0·99 (0·43 - 2·32), p=0·99). Results of other composite endpoints are shown in table 2.5.

The Polish FLUCAD study randomised 658 patients with angiographic evidence of coronary artery disease to receive either influenza vaccination or placebo[102]. A significant protective effect of influenza vaccination was seen against coronary ischaemic events (HR 0·54 (95% CI 0·29 - 0·99), p=0·047) after median follow up of 298 days. There was no significant effect on the other outcomes - CVD death (HR 1·06 (95% CI 0·15 - 7·56), p=0·95) or major adverse cardiac events (HR 0·54 (95% CI 0·24 - 1·21), p=0·13) - see table 2.5.

Study (year)	Study population	No. allocated to intervention or control groups	Outcome(s)	Hazard ratio (95% CI)	P value
FLUVACS (2004)[105]	1) 200 AMI patients	151 intervention/ 150 control	1) CVD death	HR 0·34 (0·17-0·71)[#]	0·002
	2) 101 elective PCI patients (no history of AMI/ unstable angina/CABG/ PCI)		2) CVD death or AMI	HR 0·59 (0·32-1·10)[#]	0·09
			3) CVD death or AMI or recurrent ischaemia leading to hospitalisation	HR 0·59 (0·40-0·86)[#]	0·004
FLUCAD (2008)[102]	658 patients with angiographically-confirmed coronary artery disease	325 intervention/ 333 placebo	1) CVD death	HR 1·06 (0·15-7·56)	0·95
			2) 'MACE'*	HR 0·54 (0·24-1·21)	0·13
			3) Coronary ischaemic event[†]	HR 0·54 (0·29-0·99)	0·047

Table 2.5 Randomised controlled trials of the effect of influenza vaccination for prevention of coronary heart disease

[#] Hazard ratios for results of follow up at one year (combined for AMI and elective PCI patients)
*MACE = major adverse cardiac event (cardiovascular death, AMI, coronary revascularisation)
[†] Coronary ischaemic event = MACE or hospitalisation for myocardial ischaemia

2.5.5.iii Meta-analysis of intervention studies

Pooled results from the two randomised controlled trials for 476 vaccinated participants and 483 unvaccinated controls suggested a reduction in cardiovascular death in the vaccinated group (figure 2.2). There was some evidence of heterogeneity between trials (I^2=61%, p=0·08). The fixed effects model showed a significant protective benefit - RR 0·39 (95% CI 0·20 - 0·77), but given the heterogeneity, the random effects model may provide a better estimate. For this model, the confidence interval was wider and the estimated protective effect less marked - RR 0·51 (95% CI 0·15 - 1·76). For the outcome AMI, no significant effect of influenza vaccination was seen in either model - RR 0·85 (95% CI 0·44 - 1·64) (figure 2.3).

Figure 2.2 Pooled analysis of the effect of influenza vaccination on risk of CVD death

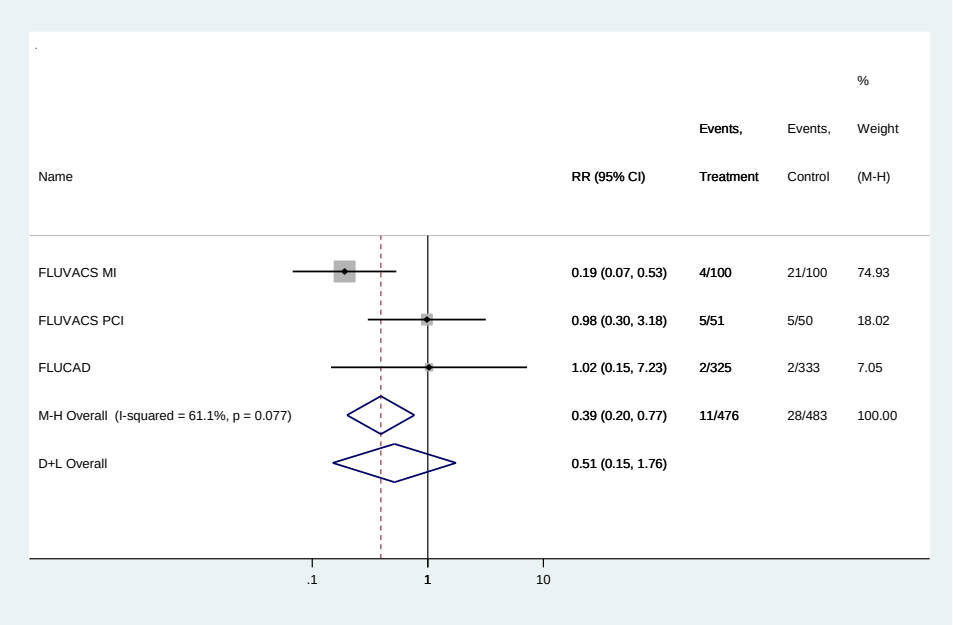
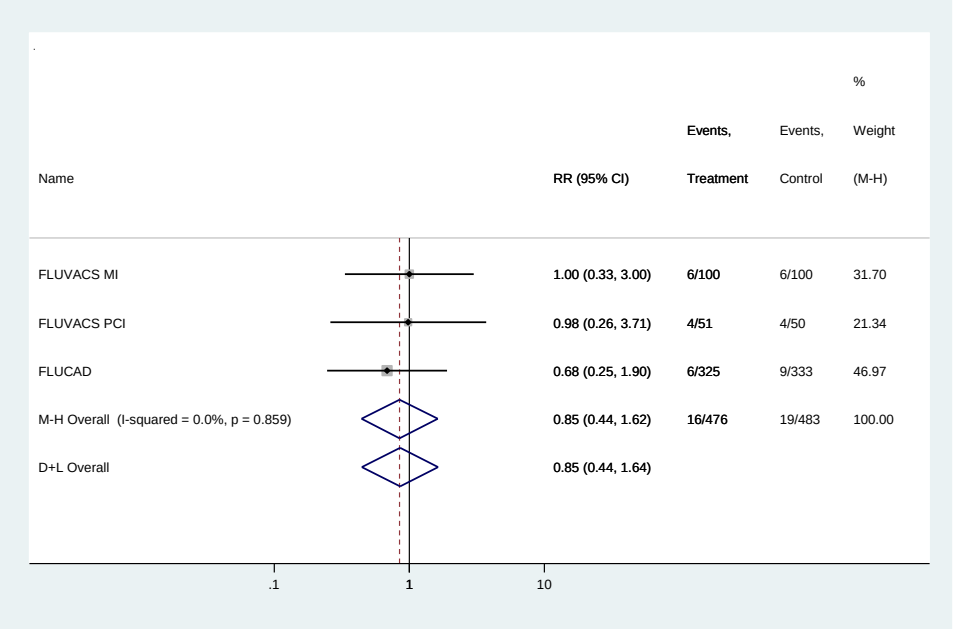


Figure 2.3 Pooled analysis of the effect of influenza vaccination on risk of AMI



2.5.5.iv Limitations of vaccine studies

Observational studies

As well as general methodological issues detailed previously for case control and case only studies, studies of influenza vaccine using any observational design are likely to be biased as to who receives vaccine. Typically vaccinated patients are healthier than their unvaccinated counterparts, which tends to lead to overestimation of vaccine effectiveness[108]. Two of the three included cohort studies attempted to control for this 'frailty selection bias' by examining the seasonality of effects of influenza vaccination on CVD death (whereby any protective effect would be expected to be greatest during the influenza season)[95,96]. One of these studies also used a novel approach to confounding by avoiding direct comparisons of mortality between vaccinated and unvaccinated people, instead comparing responses to circulating influenza in both groups[96]. It is likely, however, that residual confounding may have affected the other two cohort studies that obtained information on clinical and behavioural variables from medical records[95,97].

Interventional studies

Randomised controlled trials provide the best evidence for a protective effect of influenza vaccine against adverse cardiovascular outcomes, and indirectly for the triggering effect of influenza on cardiovascular disease. Nonetheless the two trials described were not large and few cardiovascular events (39 cardiovascular deaths and 35 AMIs) actually occurred. Methods for randomisation and allocation concealment in the FLUVACS study were unclear (though this would be less likely to affect hard outcomes such as cardiovascular death); neither study investigated the occurrence of influenza in participants. In a recent Cochrane systematic review[104], the risk of bias was described as 'moderate' for FLUVACS but low for 'FLUCAD' using the scoring system in the Cochrane Handbook for Systematic Reviews of Interventions. In addition both trials involved patients with established cardiovascular disease, which may limit the generalisability of findings to other groups. The Cochrane review concluded that, despite the significant protective effect of influenza vaccination seen against CVD death and some composite outcomes, pooled data from these two RCTs were insufficient to evaluate the effectiveness of influenza vaccine on cardiovascular events[104].

2.6 Discussion

2.6.1 Summary of findings

A range of observational studies undertaken in different settings have generally tended to support the hypothesis that acute respiratory infections - and influenza in particular - can trigger AMI. There is also more limited evidence for an adverse effect on cardiovascular death. Two relatively small randomised trials suggest that influenza vaccination reduces the risk of cardiovascular death and some coronary ischaemic events.

2.6.2 Study strengths

This review used a systematic, transparent search strategy, considering studies in all languages and from all time periods for inclusion. I used focussed outcome measures 'myocardial infarction' and 'death from cardiovascular disease', which

would tend to increase specificity of findings. I considered several graded definitions of influenza (laboratory-confirmed influenza, influenza-like illness and acute respiratory infection) as well as examining influenza vaccine studies to include evidence from a range of study types. Examining effects across different populations using a range of methodologies would tend to increase the strength and generalisability of findings as well as limiting the effect of biases intrinsic to individual study designs. This heterogeneity meant that it was, however, not possible to produce a combined estimate of effect across all studies.

2.6.3 Study limitations – publication bias

Some degree of publication bias (whereby studies with negative findings are less likely to be reported) may have affected this review. Searches of clinical trial registries (including the WHO International Clinical Trials Registry, the Cochrane Central Registry of Controlled Trials and http://clinicaltrials.gov) for unpublished trials revealed only two extra completed trials of the effect of influenza vaccine on cardiac outcomes in populations with CVD, both of which were completed in 2008 but were yet to be published (at the time of this review in 2009). Formal tests of publication bias in observational studies were not possible because we did not use meta-analysis; however it is notable that the larger and better quality studies tended to produce positive results.

2.6.4 Study limitations – issues with study quality

Some quality issues with included studies have been highlighted already in relevant sections. These included the lack of power of several smaller studies, especially earlier case control studies, to detect any effect of influenza on the development of AMI. Several case control studies were also limited by poor selection of controls: using controls groups such as those admitted to hospital for other cardiac conditions, might potentially introduce selection bias if their reason for admission was also influenced by influenza. Defining influenza for research purposes is difficult: clinical definitions, especially involving participant recall, were likely to have been affected by recall bias; triangulating information from several sources eg general practice records, laboratory data and symptoms

tended to produce more reliable results. Both ecological and early individual observational studies tended not to control for potential confounding factors, though later studies produced more robust effect estimates using multivariable analysis. While I did not use a quality scoring system, scoring systems for the quality of observational studies have not been widely validated or agreed. Included studies were critically evaluated by design according to STROBE guidance, and particular limitations highlighted throughout.

2.6.5 Interpretation of findings

Despite limitations of individual studies, results tended to be consistent across different populations in different time periods (encompassing the effects of several different circulating influenza strains). The most statistically powerful studies, eg those performed in large primary care databases, were able to show that the increased risk of AMI following respiratory infection was transient. This is in keeping with the hypothesis that influenza may act as an acute inflammatory and pro-coagulant stimulus through mechanisms such as transient alteration of endothelial function[109,110]. Only a few ecological studies attempted to quantify the population impact of influenza on cardiovascular disease by reporting a percentage of excess influenza deaths attributable to CVD. Almost all studies covered time periods of seasonal influenza circulation and some also included CVD deaths occurring in years of pandemic influenza. Though figures varied substantially between studies, partly because of varying case definitions and differential influenza circulation across years, even the lowest reported figures for excess CVD deaths were large; it was estimated that 18% of excess deaths in the USA in the 1918 influenza pandemic were due to cardiovascular disease[29].

2.6.6 Implications for policy and practice

Though this review has focussed on influenza, a large body of literature suggests that a range of acute and chronic bacterial and viral infections may be associated with increased AMI risk[111]. Influenza remains an important focus, however, because of the potentially important clinical and public health impact. Not only is influenza one of the most commonly occurring respiratory infections, but it is the

only viral respiratory infection for which there is effective available prophylaxis. Currently annual influenza vaccination is recommended in many countries for those with chronic medical conditions including established cardiovascular disease and diabetes but not for individuals with other cardiac risk factors such as hypertension[112,113]. Uptake of influenza vaccination is suboptimal, especially in those with chronic diseases. Findings from this review highlight the need to encourage vaccine uptake wherever indicated, especially in those with diabetes (which increases AMI risk) and existing cardiovascular disease, as recommended by the American Heart Association/ American College of Cardiology Foundation[114]. There is some evidence that antiviral drugs (amantadine, oseltamivir and zanamivir) prevent influenza and lessen its severity[115]. Limited observational evidence suggests that antiviral drugs may be protective against adverse cardiovascular outcomes in people with influenza infection[116–118] but no study of antiviral drugs in influenza met inclusion criteria for this review. Clear evidence of benefit from prospective trials is lacking; therefore the potential role of these drugs in preventing vascular events among high risk groups is uncertain.

2.6.7 Future directions

From these studies it is unclear whether influenza is more likely to trigger AMI than acute respiratory infections caused by other organisms. The size of any effect seen varies across existing studies (with some studies failing to demonstrate an effect). The public health impact of influenza on numbers of AMIs remains unknown. Current studies also fail to provide a definitive answer about whether influenza vaccine is effective at reducing the risk of cardiac events. Of particular importance for policymakers will be a clear definition of the population most at risk from influenza-associated AMIs: at present it is not certain whether the effect is confined to those with existing cardiovascular disease. In chapter 3 I aim to address some of these questions by investigating the effect size and impact in two different settings and populations (England & Wales and Hong Kong) using a range of data sources and methods to control for confounding.

SUMMARY

- A systematic literature review and meta-analysis of trial evidence was done focussing on the relationship between influenza (including influenza-like illness, acute respiratory infection or influenza vaccination) and AMI or cardiovascular death
- 39 studies were identified: 17 ecological, 20 individual observational studies and 2 RCTs of influenza vaccine as protection against adverse cardiac outcomes from a range of locations and time periods
- In general studies tended to support the hypothesis that acute respiratory infections - and influenza in particular - can trigger AMI, with more limited evidence for an association with cardiovascular death. The effect size and population impact remain unclear.
- Two small RCTs suggested that influenza vaccination reduces the risk of cardiovascular death and some coronary ischaemic events in people with existing cardiovascular disease, though results from pooled analysis failed to reach statistical significance.

2a. Appendix to chapter 2

2.7 Update: studies published since this review

In addition to my own work, seven studies published since this review was undertaken and fulfilling the original inclusion criteria were identified in a literature search performed on 12th October 2012. These were 2 ecological studies examining the association between influenza virus circulation and cardiovascular mortality and 5 studies (1 randomised controlled trial and 4 observational studies) of the effect of influenza vaccination on cardiac outcomes.

2.7.1 Ecological studies

One ecological study in Colombia[119] presented incidence rate ratios to compare rates of cardiovascular mortality in people aged ≥60 years during times of peak influenza circulation with the rest of the year in the time period 1997-2005. Excess influenza-related CVD mortality was quantified as an IRR 1.08 (95% CI 1.06 - 1.11) from Poisson regression models that controlled for autocorrelation but not for other environmental or temporal confounders.

In an ecological study from Hong Kong[120], linear regression models were used to estimate influenza-related excess cause-specific mortality using the difference between estimated mortality rates in the presence or absence of recorded influenza activity between 1998 and 2009. Influenza was associated with an average of 2.0 deaths from cardiovascular disease per 100,000 person years after controlling for calendar time, temperature, absolute humidity, RSV activity, a one week lag between influenza and cardiovascular death and a covariate to account for the transition in coding system from ICD-9 to ICD_10. In this study, 18% of influenza-associated excess deaths were due to cardiovascular causes.

Although methods used were disparate, with variable control for environmental confounders, both studies provide further evidence of the contribution of influenza to cardiovascular mortality across different populations and time periods.

One further modelling study, carried out to assess influenza-related excess global mortality during the first 12 months of circulation of the 2009 influenza A H1N1 pandemic virus[121], estimated that 83,300 cardiovascular deaths (46,000-179,000) were attributable to pandemic influenza. These accounted for 29% of influenza-attributed excess mortality. As this estimate was modelled, however, from crude respiratory mortality rates associated with pandemic influenza multiplied by the ratio of excess deaths from respiratory and cardiovascular diseases rather than using primary data it did not meet formal inclusion criteria.

2.7.2 Individual-level observational vaccine studies

Four individual-level observational studies used different study designs to examine the effect of influenza vaccination on AMI (3 studies)[122–124] or major adverse vascular events including cardiac death and nonfatal AMI (1 study)[125].

In a self-controlled case series study examining risk of AMI after influenza vaccination in a UK primary care population[122] a reduction in AMI was seen in the first 60 days after vaccination, greatest in the first 14 days - season-adjusted IR 0.68 (95% CI 0.60 - 0.78). There was a somewhat lower incidence ratio for early vaccinations (1st Sept – 15th Nov) compared to late vaccinations. A protective effect did not continue past 60 days but this may have coincided with the end of the influenza season.

In a prospective cohort study conducted in elderly people in Hong Kong dual vaccination with influenza and pneumococcal vaccination was associated with a reduced incidence of AMI compared to no vaccination (HR 0.52 (95% CI 0.38 - 0.71))[123]. Influenza vaccination alone did not produce a significant effect – HR 0.85 (95% CI 0.59 - 1.33) although numbers of cardiac events were small.

A large prospective cohort study nested within two global RCTs (the Ongoing Telmisartan Alone and in Combination With Ramipril Global EndPoint Trial (ONTARGET) and the Telmisartan Randomized Assessment Study in ACE Intolerant Subjects with Cardiovascular Disease (TRANSCEND) trials) found a reduction in major adverse cardiovascular events in people who received

influenza vaccination – overall adjusted OR 0.65 (95% CI 0.58 - 0.74)[125]. This estimate varied by influenza season and was significant for three seasons in which there was a good match between vaccine antigen and circulating strains but not the fourth (2003/04) when the match was incomplete. There was, however, evidence of protection outside the influenza season.

Finally, a matched case control study conducted in a UK primary care database found a reduction in odds of AMI in people who had received influenza vaccination within the last year – adjusted OR 0.81(95% CI 0.77 - 0.85)[124]. Early vaccination was associated with greater benefit than later vaccination (21% v 12% reduction in AMI). A protective effect did not persist after one year.

Overall these studies suggest that influenza vaccination affords some degree of protection against cardiovascular events, although effect sizes varied between studies. When health outcomes are compared between vaccinated and unvaccinated individuals in observational studies it may be difficult to disentangle the effect of healthy user bias. In situations where protective effects of influenza vaccination are not biologically plausible, for example outside the influenza season and in the first 14 days after vaccination (before a full immune response has been mounted), the possibility of residual biases is raised. This highlights the need for robust trial evidence.

2.7.3 Individual-level intervention vaccine studies

There has been one further intervention trial[126]. In a prospective randomised open with blinded endpoint (PROBE) study, 439 patients aged ≥50 years attending hospital in Thailand with acute coronary syndrome were randomised to receive influenza vaccination or not. At 12 months, the vaccinated group had significantly fewer major adverse cardiovascular events (including deaths and hospitalisations for acute coronary syndrome, stroke or heart failure) – 9.5% versus 19.3%, adjusted HR 0.67 (95% CI 0.51 - 0.86), p=0.005. There were also fewer hospitalisations for ACS in vaccinated patients compared to controls – 4.5% versus 10.6%, adjusted HR 0.68 (95% CI 0.47 - 0.98), p=0.039. There was no significant difference in the incidence of cardiovascular death between the two

groups – 2.3% versus 5.5%, adjusted HR 0.39 (95% CI 0.14 - 1.12), p=0.088 – although the number of events was small.

2.7.4 Updated meta-analysis

When the meta-analysis was updated with results from this trial, the new pooled estimate showed a 54% reduction in risk of cardiovascular death in patients with existing cardiovascular disease who received influenza vaccination compared to controls (compared to a 49% reduction previously). The fixed effect model showed a highly significantly protective effect and in the random effects model the protective effect only just failed to reach statistical significance – RR 0.46 (95% CI 0.21 - 1.02). Using the outcome AMI the new pooled point estimate also tended towards greater protection than before (a 33% reduction in events compared to 15% previously) but the effect was still not significant in either model – RR 0.66 (95% CI 0.39 - 1.13). Results are presented in figures 2.4 & 2.5.

Figure 2.4 Updated pooled analysis of the effect of influenza vaccination on risk of CVD death

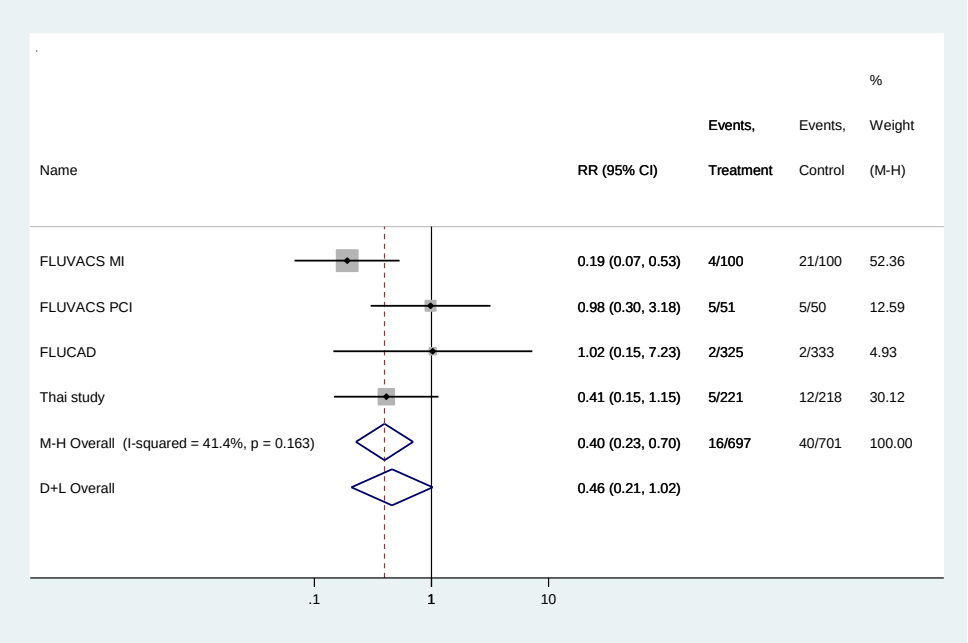
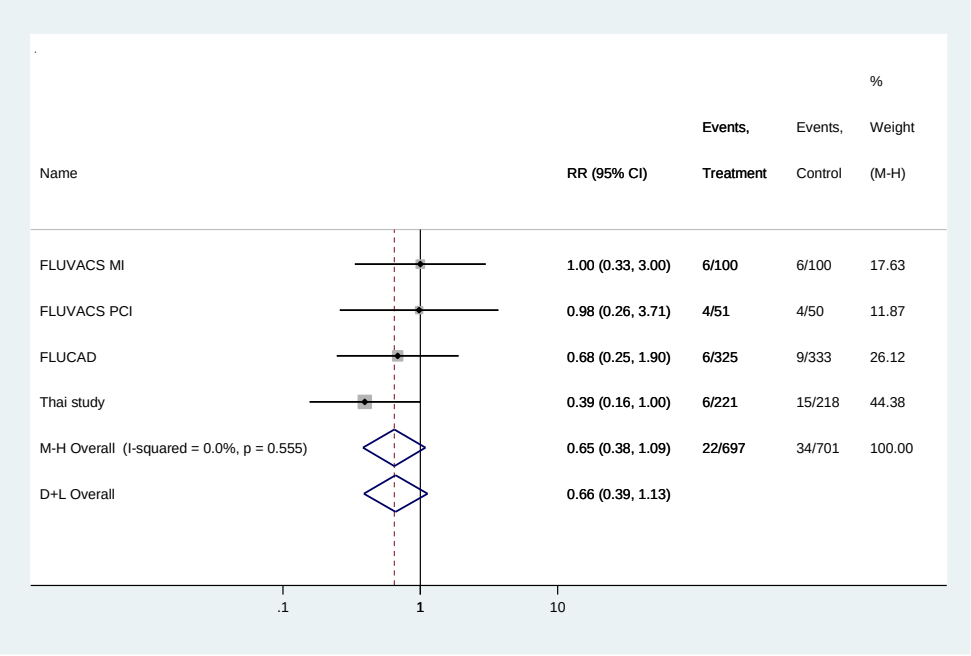


Figure 2.5 Updated pooled analysis of the effect of influenza vaccination on risk of AMI



3. Weekly time series study of influenza circulation and acute myocardial infarction in England & Wales and Hong Kong

3.1 Description of chapter contents

In this ecological time series study I investigate the relationship between influenza circulation and AMI-associated hospital admissions and deaths in England & Wales and in Hong Kong, where the summer influenza peak provides a natural experiment to examine any relationship with AMI independent of cold weather effects. Data sources include routinely collected data on hospital admissions from Hospital Episode Statistics (England) and the Hospital Authority of Hong Kong coded by International Classification of Disease codes. Similarly coded data on deaths are obtained from the Office for National Statistics (England & Wales) and Hong Kong Special Administrative Region Census and Statistics Department. Influenza data are based on ILI consultations and proportions of laboratory specimens testing positive for influenza. Poisson regression models adapted for time series are used to analyse any relationship between influenza and AMI, controlling for seasonality, long-term trends, environmental variables (weekly mean temperature and relative humidity) and lag times. Estimates are made of the proportion of AMI admissions and deaths attributed to influenza under the models in each setting.

3.2 Study rationale and introduction to data sources

3.2.1 Effects of weather conditions on AMI and influenza

A central problem in population-level studies of associations between influenza circulation and cardiovascular events is the potential for confounding by contemporaneous environmental stimuli. A J-shaped curve has been described in which extremes of temperature are associated with increased mortality from all causes including cardiovascular disease[127]. Cold weather induces various physiological changes such as increased blood pressure[128], altered levels of clotting factors, vasopressors and C-reactive protein[129] and an increase in whole blood viscosity[130]. It thus may produce a pro-thrombotic state in which

myocardial infarction is more likely to occur[131]. Hot ambient temperatures also place increased demands on the cardiovascular system and are associated with rises in mortality, especially among the elderly in whom ageing, the presence of chronic conditions and medication use may impair thermoregulation and homeostasis[132]. Climatic factors including temperature and humidity affect influenza circulation[133], with greater virus spread occurring in winter months. I therefore decided to compare data from a sub-tropical climate (Hong Kong), where winters are warmer and there is a summer as well as a winter peak in influenza circulation, with data from England & Wales to examine whether a relationship between influenza and AMI was present in either or both settings after controlling for environmental factors.

3.2.2 Clinical influenza surveillance

Both England & Wales and Hong Kong have robust surveillance systems for influenza and publish routinely collected data based on clinical and microbiological definitions. These datasets are described below to inform choices of surveillance data used in the study. Influenza surveillance in the United Kingdom is co-ordinated through the Health Protection Agency Influenza Surveillance Section of the Respiratory Disease Department. Traditionally, clinical surveillance has occurred through sentinel GP practices reporting weekly rates of consultations for influenza-like illness per 100,000 GP-registered persons. The Royal College of General Practitioners (RCGP) Weekly Returns Service has a network of approximately 100 GPs reporting throughout England & Wales; there are also separate schemes for other countries in the UK. These data are now supplemented by various telephone and internet-based surveillance schemes including the NHS Direct Syndromic Surveillance Scheme, the National Pandemic Flu Service (in 2009/2010 only), FluSurvey (an online survey of influenza in the general population co-ordinated through the London School of Hygiene & Tropical Medicine since 2009) and a community influenza telephone survey for the season 2011/2012 (run by the Health Protection Agency). There is also a Medical Officers of Schools Association scheme, in which 42 boarding schools covering a population of 12,000 children predominantly in Southern England, send weekly reports of illnesses including ILI to the Respiratory

Diseases Department at the Health Protection Agency Centre for Infections (HPA CfI). Influenza surveillance weeks run Monday to Sunday and are standardised across surveillance schemes. In Hong Kong, weekly consultation rates for ILI per 1,000 people are reported by sentinel general out-patient clinics and general practitioners and available online since 1998. Currently (in 2012) the scheme comprises data from 64 general out-patient clinics and around 50 general practitioners in private practice. Other clinical data are available on (all cause) school absenteeism rates as well as hospital admission rates for children aged 0-4 with a primary diagnosis of influenza, via the University of Hong Kong's School of Public Health Dashboard. In Hong Kong ILI is defined as fever plus either cough or sore throat, while in England & Wales there is no formal definition.

3.2.3 Laboratory influenza surveillance

Microbiological data on influenza in England arise either through one of two sentinel virological surveillance schemes or from routine laboratory reports. The HPA Virus Reference Department (VRD) / RCGP sentinel swabbing scheme involves around 85 English general practices participating in the RCGP clinical scheme. General practitioners obtain nose and throat swabs from patients presenting with symptoms of influenza-like illness. Specimens are tested at the VRD by real-time polymerase chain reaction for influenza and respiratory syncytial virus. An advantage to these data is the presence of denominators, which allows the proportion of positive specimens to be determined. A second virological surveillance scheme (the HPA CfI/ Regional Microbiology Network) is co-ordinated through a sentinel network of HPA and NHS laboratories. Routine laboratory reports on clinical specimens yielding positive results for respiratory pathogens are also reported weekly to the HPA CfI through a voluntary scheme, although data on numbers tested are not routinely available. Around 80% of influenza isolates reported in England, Wales and Scotland are sent to the HPA VRD for subtyping and antigenic characterisation. In Hong Kong, monthly summary isolate tables are published on numbers and proportions of specimens testing positive for influenza obtained from patients who presented to the GP or general out-patient clinics with ILI as well as from patients hospitalised with acute respiratory diseases.

For this study I decided to use ILI data from sentinel surveillance in primary care as well as proportions of specimens testing positive for influenza: these are well established systems with clear denominators (to enable illness rates to be calculated) and data are available dating back to the late 1990's.

3.3 Aims and objectives

Aim: to investigate the relationship between population levels of influenza circulation and hospitalisations and deaths due to AMI in England & Wales and Hong Kong.

Objectives:

1) To describe time trends in influenza circulation, and AMI-related hospital admissions and deaths for the period 1999-2008 in England & Wales and Hong Kong.
2) To generate Poisson regression models of the relationship between influenza circulation and AMI in each setting adjusting for seasonality, long-term trends, lag times and potential environmental confounders such as temperature and relative humidity.
3) To perform sensitivity analyses investigating the effect of using different methods to model environmental and temporal confounders.
4) To investigate the presence of age or gender differences in effects
5) To model whether two 'counterfactual conditions' – colon cancer and fractured neck of femur – were associated with influenza under similar conditions.
6) To estimate the proportion of AMI-related hospital admissions and deaths attributable to influenza under the models in each setting.

3.4 Methods

3.4.1 Data on AMI outcomes

I obtained data on numbers of hospitalisations and deaths associated with AMI occurring during the period January 1999 until December 2008 classified using the International Classification of Diseases, Ninth Revision (ICD-9 code 410) and Tenth Revision (ICD-10 codes I-21, I-22 and I-23). In England, I applied for hospitalisation data from Hospital Episode Statistics (the NHS Information Centre for Health and Social Care), which gave counts of AMI-associated hospitalisations based on the coded discharge diagnosis of finished consultant episodes by fiscal week of admission, age-group, sex, and region. Further information on the structure of the Hospital Episode Statistics database is given in chapter 5, section 5.2, p127. I also obtained daily mortality data on deaths from AMI or its complications by age group and sex in England and Wales from the Office for National Statistics. Equivalent AMI data for Hong Kong for the same time period were obtained from the Hospital Authority of Hong Kong (hospitalisations) and the Hong Kong Special Administrative Region Census and Statistics Department (deaths). I then aggregated daily numbers of hospitalisations and deaths associated with AMI in both regions by influenza surveillance week. I calculated age-standardised rates of AMI with reference to the World Health Organisation World Standard Population. Hospitalisation data for two counterfactual conditions – colon cancer (ICD-9 codes 153 and 154; ICD-10 codes C18, C19 and C20) and fractured neck of femur (ICD-9 820; ICD-10 S72.0 and S72.1) chosen due to its increased incidence in winter – were also obtained for both settings.

3.4.2 Data on influenza circulation

Weekly influenza surveillance data were obtained for both settings. In England and Wales, these were weekly rates of GP consultations for ILI from the RCGP network for the time period 1999–2008. Weekly proportions of nose and throat swabs testing positive for influenza virus during the influenza season (week 40–week 20) over the same time period were also obtained from the HPA/RCGP swabbing scheme. In Hong Kong, I obtained weekly rates of ILI consultations per

1000 persons reported by sentinel general practitioner and general out-patient clinics (GOPC) from the Centre for Health Protection. Laboratory surveillance data obtained from the same source comprised monthly proportions of specimens that tested positive for influenza. I used linear interpolation to generate weekly proportions of specimens testing positive from these monthly data. In sensitivity analysis I also interpolated monthly proportions of influenza virus–positive specimens to weekly proportions using spline functions.

3.4.3 Data on environmental variables

I requested data on daily minimum, mean, and maximum temperatures for the time period of the study from the Meteorological Office Hadley Centre Central England Temperature dataset via the British Atmospheric Data Centre. Central England temperatures are representative of a roughly triangular area bounded by Bristol, Lancashire, and London. I used the MIDAS Land Surface Observation Stations dataset (also obtained from the British Atmospheric Data Centre) to provide daily data on relative humidity for an approximately equivalent area (incorporating weather stations in Somerset, Lancashire, and London). For Hong Kong, daily data on minimum, mean, and maximum temperature and mean daily relative humidity were obtained from the Hong Kong Observatory. I then calculated the mean of each daily temperature and humidity parameter across influenza surveillance weeks for each setting.

3.4.4 Statistical analysis

I modelled the weekly number of acute myocardial infarction-related events (either hospitalisations or deaths) in each setting using a Poisson regression model with a scale parameter set to the Pearson X^2 statistic divided by the residual degrees of freedom to model over-dispersion[134]. I adjusted for long-term trends in rates of AMI-associated hospitalisations and deaths using both a linear and quadratic term for calendar year. This enabled only acute effects of the exposure (influenza circulation) on the outcome (AMI) to be assessed. In Hong Kong, data from 2003 were excluded from analysis, because the 2003 outbreak of

severe acute respiratory syndrome (SARS) substantially affected both health-seeking behaviour and the reliability of reporting.

In the main model I controlled for seasonal changes in AMI patterns using Fourier terms with 6 harmonics per year. In the Fourier approach, regular seasonal cycles are modelled as a linear combination of pairs of sine and cosine terms (harmonics) of varying wavelengths. Different numbers of harmonics were assessed initially and the model with the lowest Akaike Information Criterion (AIC) chosen as the final model[135]. Second, in sensitivity analysis I modelled AMI seasonality through simple stratification by month in place of Fourier terms. Third I modelled both seasonality and long-term trends using spline functions; a spline function has a flexible shape, with smoothness determined by the number of knots (or breakpoints) within splines, and is useful for modelling unknown and potentially variable seasonal and long term patterns[136]. In the spline models a judgement was made about the most appropriate number of knots per year to include. This was guided by the AIC and based on a balance between providing adequate control for potential confounders whilst avoiding generating large numbers of parameters and leaving insufficient information from which to estimate effects of influenza. Adequate seasonal adjustment allows variation in AMI-associated events explained by seasonality to be removed, allowing better assessment of the effect of the exposure (influenza).

The primary exposure was weekly levels of influenza. In England and Wales, weekly GP consultations for ILI were used to represent circulating influenza: in temperate zones, weeks with highest ILI rates correspond to or closely track weeks with the highest proportion of samples testing positive for influenza virus[137]. ILI data were also available throughout the year whereas virus data were only available in weeks 40-20. In contrast in Hong Kong, the primary measure of influenza used was weekly proportion of specimens testing positive for influenza virus: patterns of influenza seasonality are less clear in subtropical climates, so ILI data are thought to be less specific for influenza than in temperate zones[138]. Influenza surveillance data are potentially affected by delays in both consulting and reporting and thus may lag behind the true community incidence of infection. Therefore, I performed separate regressions with the exposure

variable lagged up to 4 weeks in either direction. Results are presented as an incidence rate ratio (IRR) for AMI-associated hospitalisation or death for a 10th–90th percentile change in influenza circulation. Final models were chosen with reference to the lowest AIC.

Models also included mean weekly temperature and relative humidity, both modelled as 4-knot natural cubic splines to allow for non-linearity. Sensitivity analyses included use of weekly mean temperature modelled as a linear term and as a low threshold effect, with the cut-off based on graphs showing where the predicted risk ratio of AMI-associated death or hospitalisation rose to >1, and use of daily minimum and maximum temperatures averaged separately by week and included in models as natural cubic splines. I examined the partial autocorrelation function to investigate the presence of any residual autocorrelation. All models were fitted with a term for residuals lagged by 1 week, because some degree of autocorrelation at a lag of 1 week remained after adjusting for yearly and seasonal patterns.

I repeated analyses using two different outcomes unlikely to be associated with influenza circulation: hospital admissions for colon cancer and fractured neck of femur in both settings. I also conducted an exploratory analysis to examine the relationship between influenza and AMI by age and sex. Finally, I calculated the proportion of AMI-related events attributed to influenza by predicting the number of AMIs under the final model (X) and under a model assuming zero circulating influenza (Y) as (X -Y)/X. This calculation was repeated for weeks of high influenza circulation (≥90th percentile of ILI consultations or proportion of specimens testing positive). All analyses were performed using Stata (*Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP).

3.5 Results

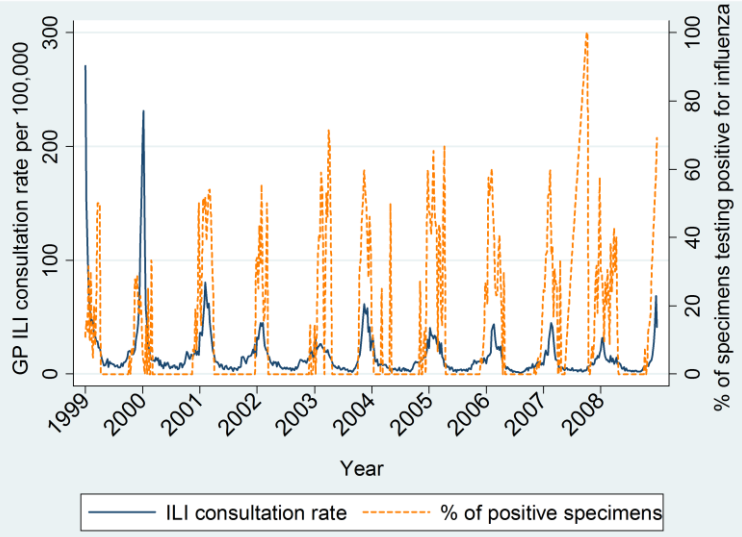
3.5.1 Descriptions of AMI and influenza patterns

3.5.1.i England & Wales

Between January 1999 and December 2008, there were 1,219,150 AMI-associated hospitalisations (median 2421 per week; interquartile range (IQR) 2112 - 2578) in England, of which 62.5% occurred in male patients. The median weekly age-standardised rate was 2.81 cases per 100,000 persons. Over the same time period 410,204 AMI-associated deaths (median 777 deaths per week; IQR 639 - 908 deaths per week) were reported in England and Wales. Both AMI-associated deaths and hospitalisations demonstrated a marked winter peak.

GP consultation rates for ILI varied from 0.8 to 270.8 consultations per 100,000 persons per week (mean 16.2 consultations per 100,000 persons per week) and were highest in 1998–1999 and 1999–2000, corresponding to circulation of the A/Sydney/5/97 strain of influenza A H3N2 subtype. ILI consultations showed a similar distribution to the weekly percentage of specimens testing positive for influenza virus during the influenza season, which ranged from 0% to 100% (mean 18.1%). Figure 3.1 below shows that seasonal peaks in ILI consultations during the study period correlated well with the proportion of specimens testing positive for influenza.

Figure 3.1 GP consultation rates for ILI in England & Wales compared to the proportion of specimens testing positive for influenza from 1999 to 2008

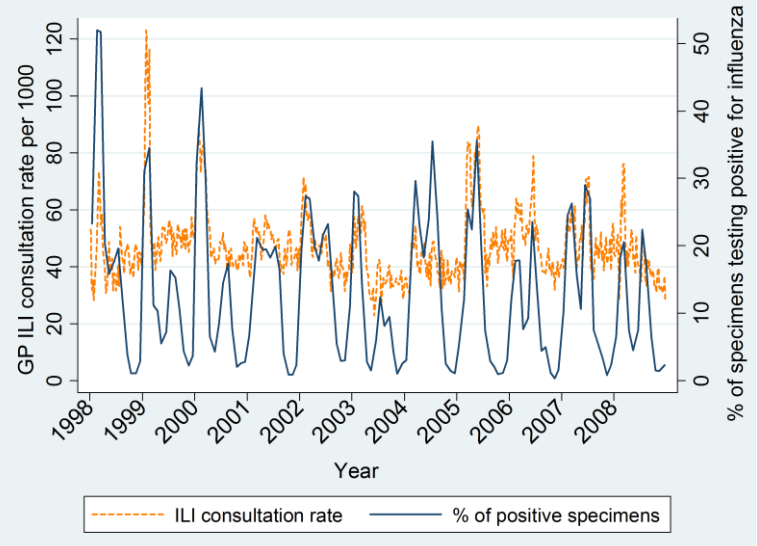


3.5.1.ii Hong Kong

In Hong Kong, during the period from January 1998 to December 2008, there were 65,108 AMI-associated hospitalisations (median 110 per week; IQR 97 - 126), equating to a median weekly age-standardised rate of 1.11 cases per 100,000 persons. 59.6% of these occurred in male patients. There were also 18,780 AMI-associated deaths (median 32 deaths per week; IQR 27 - 38 deaths per week). There appeared to be a large winter peak, as well as a smaller summer increase in the number of AMIs.

The percentage of specimens testing positive for influenza virus (measured throughout the year) varied from 0.3% to 51.9% (mean 13%) per week. Figure 3.2 demonstrates the weaker correlation between clinical ILI and laboratory isolation rates in Hong Kong compared to England & Wales. Table 3.1 describes ILI consultations, proportions of specimens testing positive for influenza and additional descriptions of exposure variables such as mean temperature and relative humidity in both settings.

Figure 3.2 GP consultation rates for ILI in Hong Kong compared to the proportion of specimens testing positive for influenza from 1998 to 2008



Variable	No. of weeks	Mean (SD)	Min	P10*	P25	P50	P75	P90	Max
Influenza – England & Wales									
GP consultation rate for ILI (per 100,000)	520	16.2 (24.1)	0.8	3.0	5.1	9.5	18.2	34.2	270.8
Specimens testing positive for influenza (%)	325†	18.1 (20.9)	0	0	0	9.6	33.3	50	100
Meteorological variables – England & Wales									
Mean temperature (ºC)	520	10.4 (4.8)	-0.1	4.2	6.4	10.2	14.5	16.7	22.0
Relative humidity (%)	520	80.6 (5.3)	61.8	73.4	76.8	81.1	84.6	87.2	93.6
Influenza – Hong Kong									
GP consultation rate for ILI (per 1,000)	571	47.1 (12.4)	22.9	35.0	38.9	45.3	52.0	60.3	123.0
GOPC consultation rate for ILI (per 1,000)	571	5.4 (2.6)	1.0	2.8	3.7	4.8	6.5	8.3	19.7
Specimens testing positive for influenza (%)	570	13.0 (10.3)	0.3	1.8	4.1	10.7	19.4	27.0	51.9
Meteorological variables – Hong Kong									
Mean temperature (ºC)	571	23.6 (4.8)	11.4	16.9	19.7	24.7	27.7	29.0	30.5
Relative humidity (%)	571	78.0 (7.9)	40.6	66.9	74.3	79.4	83.4	86.6	93.6

Table 3.1 Description of influenza and meteorological variables in England & Wales and Hong Kong over the study period

*'P10' refers to 10th percentile, 'P25' to 25th percentile etc

† England & Wales laboratory data on proportion of specimens testing positive for influenza only available during the influenza surveillance season (weeks 40 – 20)

3.5.2 Modelling AMI seasonality and long-term trends

Table 3.2 shows the number of parameters and AICs associated with various different ways of modelling seasonality and long-term trends in AMI deaths in both settings. The main three types of model for seasonality shown are a) models with an indicator variable for month, 2) models with Fourier terms and 3) models using spline functions. Three models, highlighted in bold, represent the models judged to be the 'best fit' for each category based on the lowest AIC achieved without excessively increasing the number of parameters (and 'over-fitting' models to the data).

Contents of model	Hong Kong			England & Wales		
	No. of parameters	AIC	AIC (icomp)	No. of parameters	AIC	AIC (icomp)
Monthly term (i.month)	11	6.71	3830	11	43.66	22700
Month/year term (i.monthyr)	131	6.64	3788	119	11.17	5805
Monthly term & linear year term & linear year term²	**13**	6.70	3824	13	12.18	6332
Fourier terms n=1 & linear year	3	6.76	3859	3	12.58	6539
Fourier terms n=2 & linear year	5	6.67	3809	5	12.09	6285
Fourier terms n=3 & linear year	7	6.68	3812	7	12.01	6242
Fourier terms n=4 & linear year	9	6.68	3813	9	11.83	6148
Fourier terms n=5 & linear year	11	6.67	3808	11	11.67	6068
Fourier terms n=6 & linear year	13	6.68	3810	13	11.61	6036
Fourier terms n=6 & linear year term & linear year term²	**14**	6.66	3802	14	11.58	6018
Spline (knots=0.7 per year)	10	7.17	4094	9	18.56	9654
Spline (knots=0.8 per year)	11	7.16	4091	10	18.46	9597
Spline (knots=0.9 per year)	12	7.15	4085	11	18.23	9481
Spline (knots= 1 per year)	13	7.14	4076	12	18.02	9370
Spline (knots= 2 per year)	24	6.91	3945	22	15.61	8116
Spline (knots= 3 per year)	**35**	6.58	3757	32	11.92	6201
Spline (knots= 4 per year)	46	6.52	3721	42	11.67	6071
Spline (knots= 5 per year)	57	6.49	3707	52	11.29	5873
Spline (knots= 6 per year)	68	6.51	3719	62	11.15	5798
Spline (knots= 7 per year)	79	6.53	3726	72	10.99	5715

Table 3.2 Models of seasonality and long-term trends, number of parameters and AICs for weekly AMI-associated mortality in Hong Kong and England & Wales

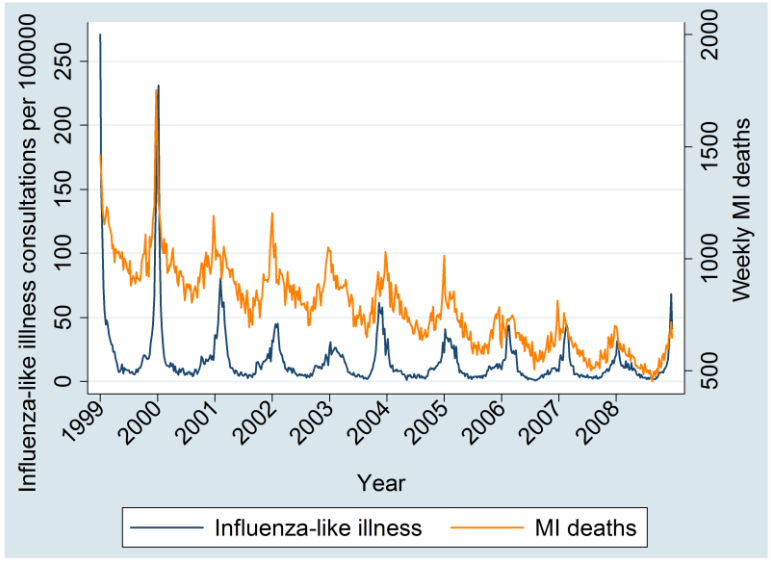
3.5.3 Associations between influenza and AMI

3.5.3.i AMI-associated deaths

Graphs of seasonal patterns of influenza circulation and numbers of AMI-associated deaths over the study time course are shown in figure 3.3. A clear correlation between the two crude variables is demonstrated, most obviously in England & Wales, where numbers of events are higher and there is only one influenza peak per season.

Figure 3.3 Weekly influenza circulation and number of AMI-associated deaths in England & Wales and Hong Kong

3.3a England & Wales



3.3b Hong Kong

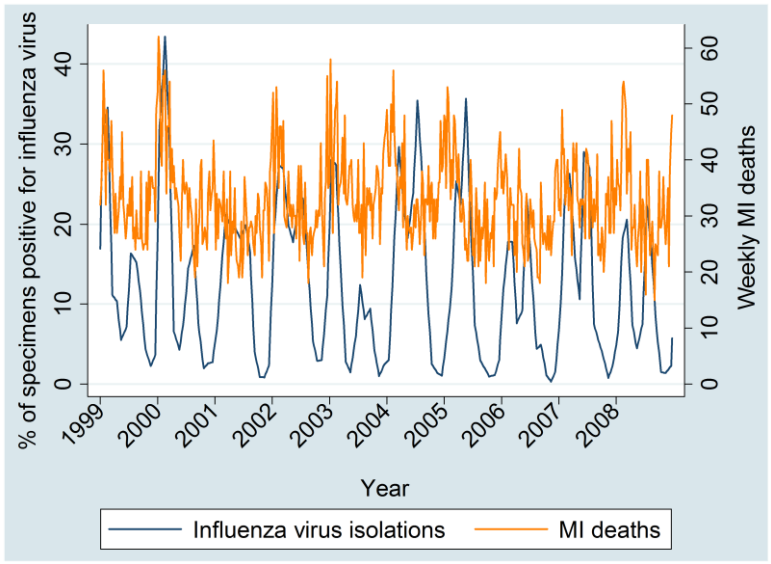


Table 3.3 (below) shows results from crude and adjusted Poisson regression analysis for both settings. These initial models assumed no lag time between reporting of influenza and AMI death. A strong association was seen between GP consultations for ILI and AMI-associated deaths in England & Wales, both before and after adjusting for environmental temperature, humidity and autocorrelation (adjusted IRR 1.036 (95% CI 1.028 - 1.043), p<0.001). In Hong Kong there was a similarly robust association between the proportion of specimens testing positive for influenza virus and AMI-associated deaths occurring in the same week (adjusted IRR 1.077 (95% CI 1.013 - 1.145), p=0.018) for a 10th–90th percentile change in proportion of positive specimens after adjusting for potential confounding factors.

Model	England & Wales			Hong Kong		
	IRR (95% CI)	p value	AIC	IRR (95% CI)	p value	AIC
Adjusted for seasonality and long-term trends	1.0359 (1.0290-1.0429)	<0.001	11.07	1.0705 (1.0043-1.1410)	0.036	6.63
As above adjusted for temperature	1.0355 (1.0287-1.0424)	<0.001	10.97	1.0723 (1.0067-1.1422)	0.030	6.60
As above adjusted for temperature & humidity	1.0355 (1.0286-1.0424)	<0.001	10.98	1.0780 (1.0121-1.1482)	0.020	6.59
As above adjusted for temperature, humidity and residual autocorrelation	1.0356 (1.0278-1.0434)	<0.001	10.71	1.0770 (1.0129–1.1453)	0.018	6.54

Table 3.3 Adjusted IRRs for the change in AMI-associated deaths associated with a 10^{th}-90^{th} percentile change in influenza circulation

In England and Wales the best fitting models included lags of either –1 week (adjusted IRR 1.051 (95% CI 1.043 - 1.058), p<0.001) for a 10th–90th percentile change in ILI consultations occurring 1 week later, or –2 weeks (adjusted IRR 1.056 (95% CI 1.049 - 1.064), p<0.001). In Hong Kong, the best model fits were seen around lag 0, with similar results given by models including lags of –1 week (adjusted IRR 1.076 (95% CI 1.012 - 1.144), p=0.02) and +1 week (adjusted IRR 1.074 (95% CI 1.010 - 1.142), p=0.023). An additional description of the lag time between ILI consultations and AMI-associated deaths is shown in figure 3.4.

Figure 3.4 Schematic illustration of the interpretation of lag times in the analysis of associations between AMI–associated death and ILI consultations. For example in the analysis with a lag time of -1 week, AMI-associated deaths in week 1 are correlated with ILI consultations in week 2, etc.

		Week				
MI		...	1	2	3	...
	lag -1	...	2	3	4	...
ILI	lag 0	...	1	2	3	...
	lag 1	...	0	1	2	...

3.5.3.ii AMI-associated hospitalisations

In England and Wales, ILI consultations lagged by –1 to –3 weeks (representing the best model fits) were associated with AMI-associated hospitalisation after adjusting for seasonality and environmental variables. There was strong evidence of a small effect: IRR for a lag of –1 week, 1.009 (95% CI 1.003 - 1.015), p=0.004; IRR for a lag of –2 weeks, 1.013 (95% CI 1.008 - 1.019), p<0.001; IRR for a lag of –3 weeks, 1.012 (95% CI 1.006 - 1.019), p<0.001. There was no association between ILI consultation rates and AMI-associated hospitalisations reported in the same week - IRR 1.002 (95% CI 0.996 - 1.003), p=0.59.

In Hong Kong, an association was seen between the proportion of influenza positive specimens and AMI-associated hospitalisations in the same week (IRR

1.066 (95% CI 1.024 - 1.109), p=0.002), after adjustment for seasonality and environmental variables. Similar model fits and results were given by models including lag times of –1 week (IRR 1.067 (95% CI 1.025 - 1.110), p=0.001) and +1 week (IRR 1.066 (95% CI 1.024 - 1.109), p=0.002). Table 3.4 shows the effect of including different lag times for influenza reporting on AMI-associated hospitalisations and deaths.

	England & Wales		**Hong Kong**	
Lag time (weeks)$^{\delta}$	**Adjusted IRR (95% CI)* for AMI hospitalisations**	**p value**	**Adjusted IRR (95% CI)† for AMI hospitalisations**	**p value**
– 4	1.006 (1.000-1.012)	0.05	1.032 (0.991-1.074)	0.13
– 3	1.012 (1.006-1.019)	<0.001	1.043 (1.002-1.086)	0.04
– 2	1.013 (1.008-1.019)	<0.001	1.057 (1.015-1.100)	0.007
– 1	1.009 (1.003-1.015)	0.004	1.067 (1.025-1.110)	0.001
0	1.002 (0.996-1.008)	0.59	1.066 (1.024-1.109)	0.002
+ 1	0.996 (0.990-1.002)	0.21	1.066 (1.024-1.109)	0.002
+ 2	0.994 (0.988-1.000)	0.04	1.059 (1.017-1.102)	0.005
+ 3	0.997 (0.991-1.003)	0.35	1.035 (0.994-1.078)	0.09
Lag time (weeks)	**Adjusted IRR (95% CI)* for AMI deaths**	**p value**	**Adjusted IRR (95% CI)† for AMI deaths**	**p value**
– 4	1.020 (1.012-1.028)	<0.001	1.010 (0.950-1.074)	0.75
– 3	1.042 (1.033-1.050)	<0.001	1.027 (0.966-1.092)	0.40
– 2	1.056 (1.049-1.064)	<0.001	1.043 (0.981-1.110)	0.18
– 1	1.051 (1.043-1.058)	<0.001	1.076 (1.012-1.144)	0.020
0	1.036 (1.028-1.043)	<0.001	1.077 (1.013-1.145)	0.018
+ 1	1.021 (1.013-1.029)	<0.001	1.074 (1.010-1.141)	0.023
+ 2	1.013 (1.005-1.021)	0.002	1.068 (1.004-1.136)	0.037
+ 3	1.010 (1.002-1.018)	0.015	1.056 (0.992-1.124)	0.089

Table 3.4 Adjusted IRRs for the change in AMI associated with a 10th-90th percentile change in influenza circulation, lagged by differing numbers of weeks in England & Wales and Hong Kong

$^{\delta}$Note a lag time of 'minus 2 weeks' refers to influenza activity occurring two weeks after AMI events in week 0, whereas a lag of 'plus two weeks' refers to ILI consultations taking place two weeks before AMI events in week 0.

*IRR for a 10th – 90th percentile change in GP ILI consultations adjusted for seasonality and long-term trends, weekly mean temperature, weekly mean relative humidity and residual autocorrelation

†IRR for a 10th – 90th percentile change in proportion of specimens testing positive for influenza adjusted for the same factors as above

3.5.4 Sensitivity analyses

Adjustments were made to the final model to test the robustness of effect estimates. Modelling AMI seasonality using alternative methods such as spline functions or indicator variables for month with a linear and quadratic term for calendar year had little effect on the magnitude and direction of influenza effect estimates. Including weekly mean temperature modelled as a linear term and as a low threshold effect gave similar results to the final model in which temperature was included as a natural cubic spline. The best model fits were seen at temperature lags of either 0 or 1 week, which gave similar results. Use of the mean of weekly maximum and then of weekly minimum temperatures modelled as natural cubic splines made little difference to effect estimates. In Hong Kong, use of the weekly percentage of positive specimens interpolated using a spline function rather than simple linear interpolation gave slightly lower point estimates but similar effects for both AMI-associated deaths and AMI-associated hospitalisations. Results of the main sensitivity analyses are shown in table 3.5.

Sensitivity analyses	AMI hospitalisations (England & Wales)		AMI deaths (England & Wales)		AMI hospitalisations (Hong Kong)		AMI deaths (Hong Kong)	
	IRR (95% CI)	p value	IRR (95% CI)	p value	IRR (95% CI)	pvalue	IRR (95% CI)	pvalue
Final model	**1.013 (1.008 -1.019)***	**<0.001**	**1.056 (1.049 – 1.064)***	**<0.001**	**1.066 (1.024 – 1.109)†**	**0.002**	**1.077 (1.013 – 1.145)†**	**0.018**
a) Seasonality								
Indicator month variable	1.016 (1.010 – 1.022)	<0.001	1.063 (1.055 – 1.071)	<0.001	1.059 (1.019 – 1.101)	0.004	1.088 (1.025 – 1.155)	0.006
Splines (3 or 5 knots per yr)	1.025 (1.016 – 1.034)	<0.001	1.063 (1.052 – 1.073)	<0.001	1.066 (1.016 – 1.118)	0.009	1.113 (1.030 – 1.201)	0.006
b) Temperature								
Linear term for mean temp	1.014 (1.008 – 1.020)	<0.001	1.057 (1.050 – 1.065)	<0.001	1.066 (1.024 – 1.109)	0.002	1.076 (1.012 – 1.145)	0.019
Low threshold effect	1.014 (1.008 – 1.020)	<0.001	1.057 (1.050 – 1.065)	<0.001	1.061 (1.019 – 1.104)	0.004	1.073 (1.009 – 1.141)	0.026
Ncs$^{\varphi}$ of maximum temp	1.014 (1.008 – 1.020)	<0.001	1.056 (1.049 – 1.064)	<0.001	1.065 (1.023 – 1.109)	0.002	1.076 (1.012 – 1.144)	0.020
Ncs$^{\varphi}$ of minimum temp	1.013 (1.007 – 1.019)	<0.001	1.056 (1.048 – 1.063)	<0.001	1.065 (1.024 – 1.109)	0.002	1.076 (1.012 – 1.144)	0.020
Ncs$^{\varphi}$ of mean temp averaged across weeks 0 and 1	1.012 (1.006 – 1.017)	<0.001	1.056 (1.049 – 1.063)	<0.001	1.067 (1.027 – 1.108)	0.001	1.074 (1.010 – 1.141)	0.021
c) % of positive specimens								
From spline interpolation of monthly data	-	-	-	-	1.058 (1.021 – 1.097)	0.002	1.048 (0.993 – 1.107)	0.090

Table 3.5 Sensitivity analyses showing effect of varying seasonality, temperature and measures of exposure in England & Wales and Hong Kong

$^{\varphi}$ Ncs = natural cubic spline

*IRR for the effect of a 10th – 90th percentile change in weekly GP ILI consultations lagged by minus two weeks on AMI, adjusted for seasonality and long-term trends, weekly mean temperature, weekly mean relative humidity and residual autocorrelation

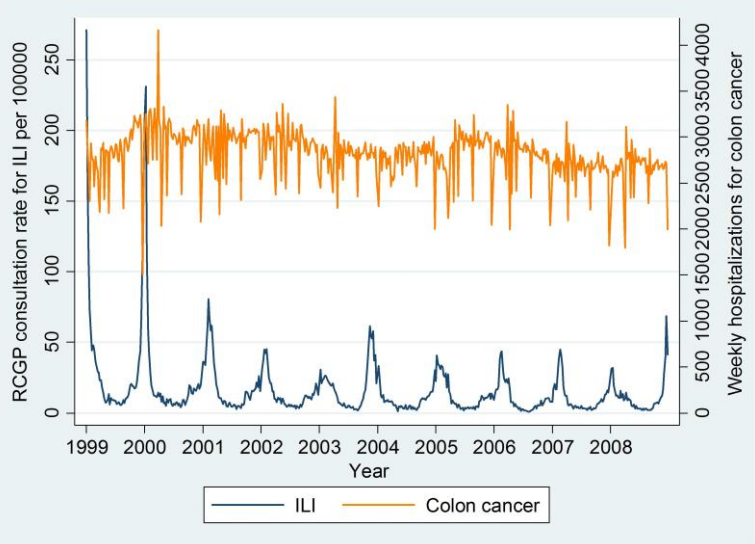
† IRR for the effect of a 10th – 90th percentile change in weekly proportion of specimens testing positive for influenza virus on AMI (with no lag), adjusted for seasonality and long-term trends, weekly mean temperature, weekly mean relative humidity and residual autocorrelation

3.5.5 Association between influenza and hospitalisations for 'counterfactual conditions', colon cancer and fractured neck of femur

In England & Wales, hospitalisations for colon cancer were not associated with influenza circulation in the same week after adjustment for these same confounders (adjusted IRR 1.005 (95% CI 0.990 - 1.021), p=0.48). Figure 3.5 shows patterns of influenza circulation and hospitalisations for colon cancer over the same time period. Investigation of lag times revealed that there was also no association between colon cancer admissions and influenza circulation lagged by up to three weeks in either direction. For the outcome fractured neck of femur, despite a peak in hospitalisations in winter months, there was no association with influenza circulation in the same week (adjusted IRR 1.009 (95% CI 0.997 - 1.021), p=0.16) or lagged by up to three weeks.

Neither hospitalisations for colon cancer (adjusted IRR 0.975 (95% CI 0.938 - 1.014), p=0.21) nor fractured neck of femur (adjusted IRR 0.994 (95% CI 0.964 - 1.024), p=0.68) were associated with influenza circulation in the same week in Hong Kong after adjustment for seasonality, long-term trends, environmental confounders and residual autocorrelation.

Figure 3.5 Weekly hospitalisations for colon cancer and ILI consultation rates in England & Wales from 1999-2008



3.5.6 Analysis stratified by age-group and gender

In both settings, the strongest associations between influenza and AMI were seen in the oldest age groups (those aged 80 years and above, and, to a lesser extent, those aged 60–79 years). In England & Wales, the adjusted IRR for AMI hospitalisations associated with influenza in the over 80s was 1.028 (95% CI 1.010-1.046), p=0.002 compared to adjusted IRR 0.965 (95% CI 0.931 - 1.000), p=0.05) in the under 40s. In Hong Kong the over 80s had an adjusted IRR of 1.161 (95% CI 1.086 - 1.240), p<0.001 compared to adjusted IRR 0.892 (95% CI 0.675 - 1.178), p=0.42 for people aged under 40 years. Incidence rate ratios for women appeared to be slightly higher than those seen for men – table 3.6.

Stratification	E&W AMI hospitalisations†		E&W AMI deaths†		HK AMI hospitalisations*		HK AMI deaths*	
	IRR (95% CI)	**P value**	**IRR (95% CI)**	**P value**	**IRR (95% CI)**	**P value**	**IRR (95% CI)**	**P value**
Overall result	1.013 (1.008-1.019)	<0.001	1.056 (1.049-1.064)	<0.001	1.066 (1.024-1.109)	0.002	1.077 (1.013 - 1.145)	0.018
Gender								
Males	1.007 (0.993-1.022)	0.303	1.049 (1.040-1.057)	<0.001	1.041 (0.993-1.091)	0.099	1.032 (0.952-1.119)	0.440
Females	1.015 (1.000-1.031)	0.045	1.064 (1.055-1.073)	<0.001	1.103 (1.043-1.165)	0.001	1.141 (1.044-1.247)	0.004
Age group								
Under 40	0.965 (0.931-1.000)	0.050	0.974 (0.890-1.065)	0.563	0.892 (0.675-1.178)	0.419	N/A	N/A
40 to 59	1.000 (0.985-1.015)	0.994	1.034 (1.016-1.053)	<0.001	0.982 (0.895-1.078)	0.706		
60 to 79	1.009 (0.995-1.023)	0.208	1.053 (1.043-1.062)	<0.001	1.057 (1.006-1.111)	0.030		
80 and over	1.028 (1.010-1.046)	0.002	1.064 (1.054-1.073)	<0.001	1.161 (1.086-1.240)	<0.001		

Table 3.6 Models of the association between influenza and AMI in England & Wales and Hong Kong overall and stratified by gender and age group. Note no age-specific data were available for Hong Kong AMI deaths.

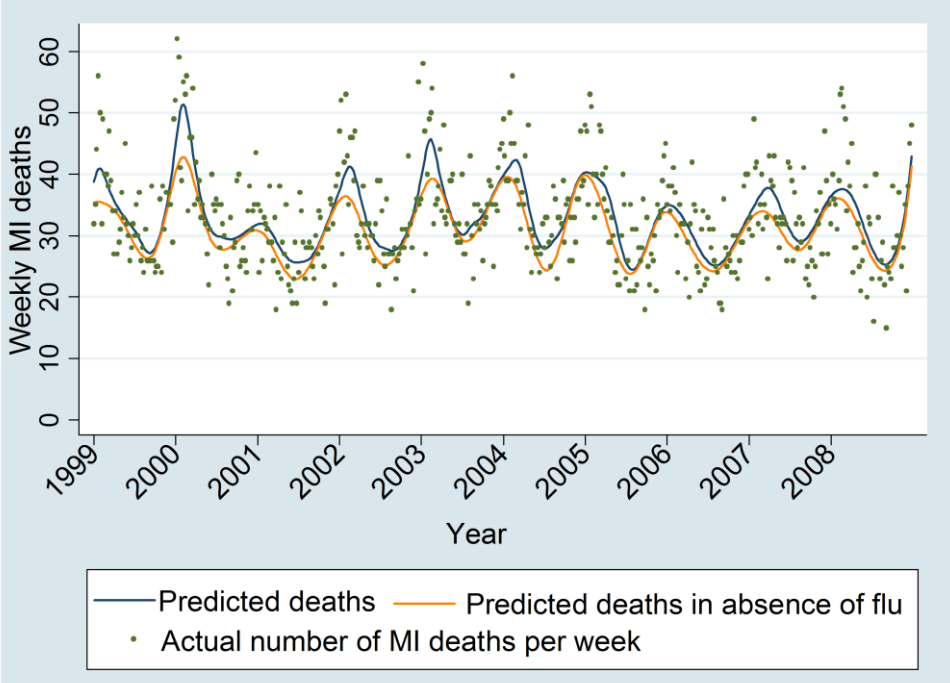
*IRR for the effect of a 10^{th} – 90^{th} percentile change in weekly GP ILI consultations lagged by minus two weeks on AMI, adjusted for seasonality and long-term trends, weekly mean temperature, weekly mean relative humidity and residual autocorrelation

† IRR for the effect of a 10^{th} – 90^{th} percentile change in weekly proportion of specimens testing positive for influenza virus on AMI (with no lag), adjusted for seasonality and long-term trends, weekly mean temperature, weekly mean relative humidity and residual autocorrelation

3.5.7 Predicted percentage of AMIs attributable to influenza

Proportions of AMI-associated deaths attributed to influenza under the final models ranged from 3.9% - 5.6% for Hong Kong and 3.1% - 3.4% for England and Wales, depending on the model of seasonality used. Proportions of AMI-associated hospitalisations attributed to influenza were smaller in both settings: 3.0% - 3.3% and 0.7% - 1.2%, respectively. In weeks in which influenza circulation was in the ≥90th percentile, 9.7% - 13.6% of AMI-associated deaths in Hong Kong and 10.7% - 11.8% of AMI-associated deaths in England and Wales were attributed to influenza. For AMI-associated hospitalisations, the corresponding figures were 7.5% - 8.2% and 2.5% - 4.6%. Figure 3.6 shows an example of excess AMI deaths expected in Hong Kong at times of peak influenza circulation.

Figure 3.6 Predicted AMI deaths in Hong Kong under the model (blue) compared to predicted deaths under a model where influenza circulation was set to zero (orange)



3.6 Discussion

3.6.1 Summary of findings

These results demonstrate strong associations between population levels of influenza and AMI-associated deaths and hospitalisations in both a temperate (England and Wales) and subtropical climate (Hong Kong) after adjustment for temporal trends and relevant environmental confounders. I estimate that a small but important proportion of AMIs in both settings may be attributed to influenza, with figures increasing in weeks of highest influenza circulation.

3.6.2 Study strengths

A strength of this study was the comparison of data from a temperate and a subtropical climate. Although most early ecological studies were done in temperate zones, it has more recently been suggested that influenza-associated mortality in warm regions such as Hong Kong is comparable to that of temperate regions[139]. I saw a slightly greater effect of influenza on both AMI-associated deaths and hospitalisations in Hong Kong versus England and Wales, but this should be interpreted with caution because population size, and therefore the number of events in Hong Kong, was much lower. Extremes of temperature are known to be associated with deaths due to cardiovascular disease[140]. The use of several methods to control for temperature and the differing relationship of influenza with temperature in Hong Kong, including the presence of a summer influenza peak, reduced the chance that residual confounding by environmental variables was responsible for effects observed. Another strength was the use of the same models with two other 'counterfactual conditions' (colon cancer and fractured neck of femur) as outcomes, which found no association with influenza, despite the increased incidence of fractured neck of femur in winter. This suggests that associations seen with AMI were not spurious and related to the modelling method. This is also the first study to estimate the influenza-attributable risk of AMI-hospitalisations and deaths: previous ecological studies have tended instead to estimate the proportion of influenza-associated excess mortality due to cardiovascular or AMI deaths.

3.6.3 Study limitations – reporting delays in routine surveillance data

Weaknesses inherent to using routine surveillance data for research include potential lack of coverage and lack of timeliness[141]. Although underreporting of influenza is common, this would tend to dilute rather than bias the direction of our results. Reporting delays are potentially more problematic. One United Kingdom–based study showed that telephone calls to NHS Direct for colds and influenza preceded GP reports of the same symptoms by 1 - 3 weeks[142]. This may explain why we saw the best model fits, and greatest estimates of effect, when UK influenza data was lagged by –1 to –3 weeks (representing our assumption that reported ILI consultations represent illness occurring in the community some time earlier). A US study found a 1 to 2 week lag between internet searches for influenza-associated information and primary care consultations for ILI[101]. A survey of 918 people with an ILI attending GPs in England showed that approximately one half of persons aged >45 years waited at least 6 days before consulting a GP, with 13% waiting for two or more weeks[143]. Although consultation delays in surveillance data are the most likely explanation, peaks in AMIs might precede GP reports of ILI if triggered by other synchronous environmental events; however, we used multiple sensitivity analyses to control for temperature.

3.6.4 Study limitations – controlling for circulating respiratory viruses

Other respiratory viruses such as respiratory syncytial virus (RSV) or human metapneumovirus may cause influenza-like illness symptoms. While their association with AMI is not known, it is possible that some of the apparent association between influenza and AMI was explained by co-circulating respiratory viruses. Although I considered controlling for other viruses, 95% of RSV samples in the United Kingdom are recovered from children[144], and RSV seasonality tends to differ from that of influenza. Human metapneumovirus is not common in adults[145] and data were not available for the time period of the study. In the United Kingdom, although ILI consultations lack specificity for influenza, the highest positive predictive values of ILI occur at times of peak influenza circulation[34]. I did not use influenza virus data, which were limited by relatively

small numbers of specimens (mean 37 per week) and confined to the influenza season. The peak week of influenza virus activity in the UK tends to precede the peak for ILI consultations by around 2 weeks[146]. In Hong Kong, where influenza virus data were used as the main exposure, I did not see the same pattern of lag times. Differences in consulting behaviour may explain the reduced reporting delay in Hong Kong influenza data. After the SARS outbreak of 2003, official advice was to consult a doctor as soon as influenza-like symptoms are experienced. In contrast, campaigns in the UK throughout the late 1990s aimed to discourage attendance at the GP for colds and ILI, to try to reduce unnecessary prescriptions of antibiotics[144].

3.6.5 Interpretation of results in context of previous findings

Similar studies have shown rises in related but less specific outcomes, such as deaths due to cardiovascular disease[30,64,71], or less sensitive outcomes, such as autopsy-confirmed AMI-associated deaths[76], during influenza epidemics. Based on the systematic literature review in chapter 2, I believe that this is the first study to examine the relationship between influenza circulation and national rates of fatal and non-fatal AMI in two different settings and populations. Compared to previous studies a more robust approach is taken to controlling for environmental temperature to minimise the risk of residual confounding. Present results are similar to the small number of previous studies that did control appropriately for temperature[71,120]. As with other ecological studies that presented results stratified by age[30,65,71,73,77], I saw associations between influenza and adverse cardiovascular events that were most marked in the oldest-aged persons, who are more likely to have extensive underlying coronary disease. In population-level analyses, however, it is not possible to ascertain whether AMI-associated events occurred in individuals with underlying cardiovascular disease. Although results showed a stronger association between influenza and AMI-associated deaths versus AMI-associated hospitalisations, it remains unclear whether influenza is likely to trigger cardiac events of greater severity. Finally, it is difficult to compare the population impact estimated in this study with previous studies as I calculated percentage of AMI deaths attributed to influenza rather than % of excess influenza mortality due to CVD, although findings are in

keeping with one previous figure of 8% from a Colombian study of seasonal influenza[119]. I considered % of AMI deaths due to influenza to be a more meaningful measure of population impact and also did not have data on other influenza-attributable causes of mortality to estimate the other figure.

3.6.6 Implications for policy and practice

Overall, up to 5.6% of AMI-associated deaths in Hong Kong and 3.4% in England and Wales were attributed to influenza (equating to 1052 and 13,947 deaths, respectively). Although this is a relatively small proportion, in England and Wales, over the study period, influenza vaccination rates among persons aged 65 years were around 65% - 75%[41]. In Hong Kong, influenza vaccine was not introduced for community-dwelling older people until 2004, with uptake estimated at 31.2% in 2004 and 48.1% in 2005[147]. The effect of influenza on AMI occurred mainly in elderly persons who – especially in England and Wales – are relatively highly vaccinated. Without access to seasonal influenza vaccine, the potential for impact on AMI events could be much greater. This study highlights the need for more effective influenza vaccines in the elderly. In this group in particular acute cardiac events should be considered when anticipating influenza outbreaks, both for health service planning and timing of health awareness campaigns. For examples health awareness messages about cardiac symptoms for the elderly, their carers and nursing home staff could be aligned with the start of laboratory influenza surveillance.

3.6.7 Future directions

I found a consistent association between seasonal influenza circulation and acute AMI-associated hospitalisations and deaths in two different settings characterized by differing populations, climates, and patterns of health-seeking behaviour. While there is fairly consistent ecologic evidence that influenza virus circulation and ILI is related to cardiovascular mortality and some individual-level evidence that acute respiratory infections may be related to AMI, further work is needed at individual level, ideally incorporating laboratory confirmation of influenza and information on underlying illnesses. Chapter 4 describes an individual-level

study based on primary care records, using data from influenza surveillance to explore this association further.

SUMMARY

- A weekly time series regression analysis was used to examine associations between population levels of influenza and AMI – associated hospitalisations and deaths in a temperate (England & Wales) and subtropical climate (Hong Kong)
- Strong associations were seen between influenza circulation and AMI outcomes in both settings after adjusting for a range of temporal and environmental confounders
- Effects were greatest in elderly individuals, especially those aged ≥80 years
- No association was found between influenza circulation and the counterfactual conditions fractured neck of femur or hospitalisation for colon cancer in either setting
- Up to 5.6% of AMI-associated deaths in Hong Kong and 3.4% in England and Wales were attributed to influenza, with figures rising in weeks of highest influenza circulation

4. Self-controlled case series analysis of acute respiratory infection and incident myocardial infarction using the General Practice Research Database

4.1 Description of chapter contents

The study described in this chapter uses individual-level primary care records from the General Practice Research Database (GPRD) to examine the association between GP consultations for acute respiratory infection and incident myocardial infarction. The GPRD is a rich resource containing virtually complete anonymised medical records on a large representative sample of around 8% of the UK population. This analysis uses self-controlled case series which is related to the cohort method but has the additional advantage of removing the need to control for fixed confounders. It allows detailed investigation of the timing of respiratory primary care consultations in relation to AMI. Effects are examined by age and gender. A preliminary investigation is done into whether acute respiratory infections judged more likely to be caused by influenza (by medical codes used to classify illness and timing of the episode in relation to circulating influenza virus) have a different effect to acute respiratory infections without these indicators of influenza.

4.2 Study rationale and introduction to data sources and methods

4.2.1 General Practice Research Database

Individual-level data are essential to avoid the risk of ecological biases associated with studying a phenomenon at population level alone. To generate adequate power for individual-level analyses I used data from anonymised electronic medical records of individuals registered at general practices contributing to the GPRD[148]. Worldwide this is the largest computerised database of longitudinal primary care records, with data currently collected on around 5.2 million active patients from approximately 630 primary care practices throughout the UK. GPRD started collecting data in 1987 but historical records can date back decades

before this. Unlike traditional data collected for research, GPRD records arise from an unselected population with effectively 100% participation. Types of information available include demographic details, clinical consultation records, referral information, results of clinical investigations and prescribing data as well as behavioural and lifestyle factors such as smoking and alcohol consumption. Further detail on the structure of the GPRD dataset in relation to this analysis is given in section 4.4.1. To access GPRD data for this study, I designed a data specification based on time period, outcome diagnosis (incident AMI) and patient characteristics - appendix 10.1.1. All records for patients fulfilling specification criteria were then extracted centrally at GPRD.

4.2.2 Quality control in GPRD

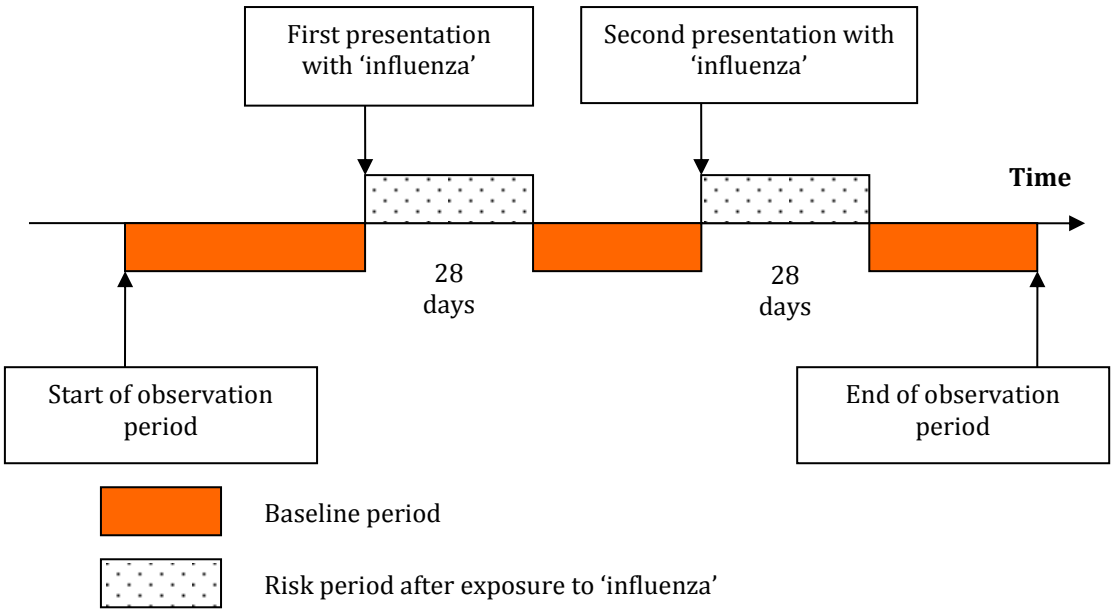
Quality control and regular auditing of data are central to GPRD. Individual patient records are labelled as 'acceptable' for use in research if various conditions relating to quality of data recording and continuity of follow up are met eg a year of birth record must be present, age should be less than 115 at the end of follow up, gender must be recorded as male, female or indeterminate (see footnote for conditions that render a patient's data unacceptable[1]). At practice level, the practice is given an 'up-to-standard' date, at which the practice is considered to have sufficient high quality continuous data for use by researchers. This is determined by analysis of the continuity of data entries and avoidance of the use of data from 'ghost' patients ie those who have died or transferred out of the practice. Diagnoses in GPRD have been well-validated in peer reviewed publications including a recent systematic review that demonstrated across 55 publications that a median of 85.3% of circulatory diagnoses (including AMI) were confirmed using both internal and external methods of validation such as questionnaires to GPs and use of manual diagnostic algorithms[149].

[1] The following conditions render the quality of patient data unacceptable for use in research: an empty or invalid first registration date; absence of a record for a year of birth; a first registration date prior to their birth year; a transferred out reason with no transferred out date; a transferred out date with no transferred out reason; a transferred out date prior to their first registration date; a transferred out date prior to their current registration date; a current registration date prior to their first registration date; a current registration date prior to their birth year; a gender other than male/ female/ indeterminate; an age >115 years at end of follow up; recorded healthcare episodes in years prior to the birth year; temporary patient registration status

4.2.3 Description of self-controlled case series

In self-controlled case series, only 'cases' are sampled ie only patients experiencing the event or outcome of interest, here AMI, are included in analysis. The effect estimate generated in self-controlled case series through conditional Poisson regression is an incidence ratio. This compares the rate or hazard of an event occurring in a period following exposure to a potential risk factor to the rate or hazard of events occurring in all other observed time periods for each individual. Figure 4.1 shows an example timeline for an individual who experiences two periods of exposure to influenza during follow up.

Figure 4.1 Graphical representation of exposure and baseline periods in a self-controlled case series timeline



4.2.4 Application and advantages of self-controlled case series

Derived from cohort logic, self-controlled case series was originally developed to investigate associations between acute outcomes and transient exposures such as risks of adverse events in time periods following vaccinations[150]. Subsequently the method has been applied more widely to diverse problems from investigating the risk of acute cardiovascular events after invasive dental treatment[151] to examining the risk of fractures associated with thiazolidinedione use [152]. The main advantage over other methods for examining the relationship between

influenza infection and AMI is that self-controlled case series controls implicitly for fixed confounders such as genetic factors, socio-economic status, gender, frailty and health-seeking behaviour[150], as comparisons are within an individual at different time points. Self-controlled case series is also the most valid method of analysis when using routinely collected data to avoid the risk that incomplete recording of information on residual confounders might compromise reliability or generalisability of results. While fixed confounders are controlled for implicitly it is still possible to include time varying confounder such as age and season in multivariable models[150]. Using people as their own controls avoids the problem of selecting an appropriate control group with the inherent risks of indication and selection bias. Self-controlled case series is statistically efficient compared to the retrospective cohort method[150].

4.2.5 Comparison of self-controlled case series, case control and cohort methods

Previous studies have compared results when data were analysed using both self-controlled case series and either case control or cohort methods. Taking antidepressant use and risk of hip fracture as an example, a case control study using GPRD suggested an increased risk of hip fracture in patients who used tricyclic antidepressants – OR 4.76 (95% CI 3.06 - 7.41) for 0-14 days after prescription[153]. An even greater risk of hip fracture was found when examining prescriptions for selective serotonin reuptake inhibitors (SSRIs), a newer class of antidepressant, for which the OR was 6.30 (95% CI 2.65 - 14.97) for days 0-14. When the same data were reanalysed using self-controlled case series, incidence ratios for the risk of hip fracture occurring over the same time period were 2.30 (95% CI 1.82 - 2.90) and 1.96 (95% CI 1.35 - 2.83) respectively[153]. This suggested that while both classes of antidepressant drug were associated with a small independent increase in hip fracture risk in the first weeks of treatment, effect sizes were artificially inflated by using the case control approach, more so in the SSRI group. This is likely to be due to residual biases eg indication bias (where the indication for the drug, here depression, is itself associated with an increased risk of falling and therefore of hip fractures) and selection bias (whereby frailer people are preferentially prescribed SSRIs as these are thought to have fewer side

effects, but frailty is associated with increased risk of hip fracture). Residual biases also appeared to inflate effect sizes when using cohort methods compared to self-controlled case series in the same dataset to examine any association between influenza vaccination and exacerbations of asthma in children[154].

4.2.6 Assumptions affecting self-controlled case series

There are several assumptions made when using self-controlled case series that are relevant to this analysis. In brief: the probability of exposure (here acute respiratory infection) should not be affected by occurrence of an outcome event (here AMI); event risk should be small over the observation period; there must be variability in the time or age of event; the length of follow up should not be influenced by occurrence of an outcome event[150]. These will be discussed further in the appropriate sections.

4.3 Aims and objectives

Aim: To investigate the association between GP consultation for acute respiratory and AMI using the GPRD.

Objectives:

1) To examine whether there was a greater incidence of AMI in time periods following GP consultation for acute respiratory infection using self-controlled case series adjusted for age and season.
2) To identify whether there was a biological gradient of risk in days following GP consultation for acute respiratory infection.
3) To investigate whether any effect varied by age and gender.
4) To explore whether acute respiratory infections that a) occurred during time periods when influenza was circulating and b) were coded with influenza-like illness codes were more likely to be associated with AMI than other illness episodes.
5) To examine the effect of inclusion of fatal episodes of AMI (as this goes against one of the assumptions on which the self-controlled case series is based).

4.4 Methods

4.4.1 Data sources

For this analysis, GPRD records were used as the source of both outcome and exposure information. A typical record contained an encrypted patient identifier variable that was consistent throughout all files and allowed GPRD information from different files to be merged. Demographic details such as sex, age and year of birth were available for each patient as well as a practice code and details of registration with the practice including dates of new registration and transfer out (if relevant). In general, details of clinical encounters in primary care eg a new occurrence of a respiratory illness or symptom are entered into the 'Clinical' files by the GP who chooses the most appropriate descriptive term for the illness from a drop down list. A corresponding Oxford Medical Information Systems (OXMIS) or Read code is also given and the date of encounter entered. AMI records typically reach a general practice through hospital correspondence eg discharge letters. The diagnosis is likely to be entered into the 'Clinical' files. Additionally, details of investigation results (such as electrocardiographic findings reflecting a myocardial infarction) could be entered into a patient's 'Test' file and information about referrals eg for further cardiac investigations following myocardial infarction may be entered into the 'Referral' file. While the dataset also comprises 'Therapy' files containing drug information coded using Prescription Pricing Authority codes as well as date, dosage and method of administration, therapy files were not used to identify either outcome or exposure events as they were felt to be too indirect and non-specific. Lastly the dataset includes various files of 'Additional Clinical Data', including records of 'Death administration' used to identify dates of death and thereby end of follow up.

4.4.2 Study population and follow up

Patients were drawn from those registered with a practice contributing data to the GPRD during the study period 01/01/1999 until 31/12/2008. Potentially eligible patients had experienced a first AMI during this period and were aged ≥40 years at the time of event. Patients entered the study either at the study start

date or at their date of registration with a GPRD practice or at the date a practice reached 'up-to-standard' status, whichever was latest. Follow up continued until the earlier of either the study end date or the date a patient left the practice (eg due to death or transferring to another general practice). According to GPRD documentation the most reliable way to identify deaths in GPRD is to have a record of leaving the practice with a 'transfer out' reason of 'Death'. It is recognised, however, that there may be a delay between a patient's actual date of death and the date that they are 'de-registered' from the practice (the median delay for all deaths in GPRD is 19 days). Therefore a list of 'Statement of Death' codes provided with the dataset by GPRD was used to extract records of deaths from 'Clinical' and 'Referral' files. In addition records were also extracted from the 'Death administration' area of the 'Additional Clinical Data' files along with dates of death. These records were appended and duplicates and those with missing or impossible dates (eg date of death before the start of the study or before AMI) were removed. The earliest recorded date of death from any of these files was taken as the end of follow up where it occurred no earlier than 95 days before the recorded date that a patient left the practice (as advised by documentation from GPRD). Otherwise if no other record of death was found but the 'transfer out reason' was 'Death', registration records were used and the end of follow up for that individual was taken as the leaving date from the practice.

4.4.3 Sample size calculations

The user-written Stata command 'sampsi_sccs' was used to calculate sample size requirements. Assuming an incidence ratio of 3 ('rho'), a duration of the post-exposure risk period of 28 days (based on findings from a previous similar study in GPRD[84]) and a duration of entire observation period of 3,304 days (based on median length of follow up), the sample size needed would be 593 to estimate results with 90% power at the 5% significance level. Reducing the IR and reducing the length of the post exposure risk period both increase sample size requirements. Table 4.1 below shows the different sample sizes required for this study under varying conditions.

Post exposure risk period (days)	Rho = 3	Rho = 2
91	193	587
28	593	1837
14	1171	3644
7	2328	7257

Table 4.1 Sample sizes needed for self-controlled case series in GPRD under different conditions

4.4.4 Generating codelists

Before extracting records for use in the analysis it was necessary to generate lists of all Read and OXMIS codes that could be used by GPs to code relevant conditions or events. The general method is described here and then additional specific details discussed in sections 4.4.5 (outcome), 4.4.6 (exposure), 4.4.8 ('influenza-like illness' codes). Taking incident myocardial infarction codes as an example, initial discussions were had and brainstorming done with a senior clinician experienced in use of general practice data (Professor Smeeth) to identify likely terms. The complete dictionary of Read codes was searched electronically for appropriate words eg 'myocardial infarction', 'heart attack', 'myocardial thrombosis' according to a Stata loop defined by Dave and Petersen[155]. Word-searches (along with the attached code) were saved in a temporary file. Similar searches were then carried out for likely codes based on code stems identified during the word searches and files were again saved temporarily. Results from word- and code-searches were merged, duplicates were removed and lists of potential terms reviewed. Irrelevant codes and those where the myocardial infarction described was not necessarily incident were removed manually. The final codelists were reviewed and agreed by the research team before being used for data extraction.

4.4.5 Outcome

Records for the first occurrence of AMI were identified with a codelist for incident myocardial infarction generated using methods as described above. Records containing these codes were extracted from the 'Clinical', 'Referral' and 'Test' files

along with the date of event and duplicates removed. In primary care data the level of detail entered on AMI varies from very specific codes (such as 'Postoperative transmural myocardial infarction inferior wall') to the very general ('Heart attack'). Therefore no attempt was made to extract further information on type of AMI in this analysis. In chapter 5 linked MINAP records were used to classify and then stratify analysis by AMI type. All AMI records had to occur at least 6 months after the start of a patient's follow up in GPRD to reduce the risk of historical recording of events. For this reason I also removed records of AMI recorded on the same day as a routine encounter eg a well person screen or new patient registration visit. The analysis was restricted to first AMI events only to maintain relative homogeneity of the sample and also because a person's risk profile changes considerably after an AMI. This may invalidate the assumption of the self-controlled case series that a person can be compared with themselves in all other time periods (as after a first event a person would be at considerably higher risk of subsequent AMI events). The first recorded date of AMI was taken to be the AMI date and any subsequent AMI records dropped. This dataset of first AMI events was then merged with demographic and registration information for each individual and date checks done to ensure that no AMI records occurred before the start or after the end of a person's follow up. In sensitivity analysis fatal events were defined as those with a date of death (based on the earliest plausible death record) within 28 days of an AMI. These records were dropped and analysis repeated. This was done to ensure that no bias was introduced through including records in which the length of follow up was dependent on occurrence of an outcome event.

4.4.6 Exposure

To be included in self-controlled case series analysis patients had to have both a record of first AMI and a record of GP consultation for acute respiratory infection occurring at any time during their follow up. A codelist was devised using methods previously described for acute respiratory infection with a systemic component eg 'acute bronchitis', 'tracheitis', 'pneumonia' designed to capture the more severe end of a spectrum of acute respiratory infections that present to general practice. These would include illnesses caused by influenza virus. Three

subsets of codes from this list were defined according to table 4.2 for use in different analyses. Episodes classified by isolated respiratory symptoms, eg sore throat, were not extracted as these were thought unlikely to represent either influenza or its complications. Records of episodes with general acute respiratory infection codes (codelist 1) were extracted from Clinical GPRD files along with the date of consultation. Where there were multiple different codes entered for the same day of illness, the first record was saved (the exact code did not matter as initially we were interested in the date only). Any records occurring within 28 days of a previous acute respiratory infection code were dropped as these were likely to refer to the same episode of illness. This dataset of respiratory records and dates was merged on patient identifier with AMI, demographic and registration records to generate the initial dataset for analysis (dataset a).

Codelist	Contents	Rationale
1	Acute respiratory infection codes with a systemic component eg 'chest infection', 'acute bronchitis'	Used to extract episodes of acute respiratory infection
2	As list 1 but minus non influenza organism-specific codes eg 'staphylococcal pneumonia'	Used to extract episodes where diagnosis could plausibly be influenza
3	Influenza-like illness codes eg 'influenza with pharyngitis'	Used to extract influenza-like illness episodes
4	As list 1 but minus influenza-like illness codes	Used to allow comparison between episodes of general acute respiratory infection and influenza-like illness

Table 4.2 Description of codelists used to extract ARI episodes

4.4.7 Influenza surveillance data

To identify infections more likely to be caused by influenza I used information on timing of influenza virus circulation from national surveillance systems. First, episodes of acute respiratory infection where the diagnosis could plausibly be influenza were extracted from Clinical records as before using codes from codelist 2. Again, duplicate records for the same day of illness were removed, as were episodes occurring within 28 days of the initial illness date. To identify weeks in

which influenza virus was circulating (and therefore in which acute respiratory infections presenting to the GP were more likely to be caused by influenza virus) weekly proportions of nose and throat specimens testing positive for influenza virus by reverse-transcriptase polymerase chain reaction covering the study period were used. These were taken from patients with ILI attending sentinel general practices in England under the HPA/ RCGP swabbing scheme (described in section 3.2.3, p72). The peak week of influenza circulation for each influenza season was defined as the week with the highest proportion of specimens testing positive if the total number of specimens was ≥20. In separate analyses, two, three and four weeks either side of this peak week were labelled as influenza circulation weeks. Separate datasets of acute respiratory infections occurring in influenza circulation weeks (dataset b) and non-circulation weeks (dataset c) were generated and records sorted and merged on patient identifier with AMI, demographic and registration records as above.

4.4.8 'Influenza-like illness' codes

I also considered that the probability of an episode of respiratory illness being caused by influenza virus may vary according to codes used by GPs to classify illness. Therefore I conducted an analysis of the effect of episodes of acute respiratory infection coded with ILI codes on AMI incidence compared to that of episodes coded with other acute respiratory infection codes. To generate datasets for this analysis episodes of ILI were extracted from Clinical files using codelist 3 (influenza-like illness codes). The first date of recording of ILI was taken as the date of the episode and any duplicate records of ILI occurring within 28 days were dropped. This dataset of ILI records and dates was merged on patient identifier with AMI, demographic and registration records to generate the ILI dataset (dataset d).

To generate a comparison dataset containing episodes coded with other acute respiratory infection codes (codelist 4), records of infections were extracted from Clinical files and full duplicates removed. A 'general respiratory infection' label was applied to each record to distinguish from ILI records in subsequent steps. After sorting by patient identifier and date, records were merged with dataset d

(which contained only ILI records). When both an ILI code and a general code were entered for a patient on the same day this episode was classified as an ILI and deleted from the dataset of 'other acute respiratory infections'. When an ILI record occurred within the same episode of illness as a general code (ie within 28 days) this was also classified as an ILI and any records related to that episode of illness removed from the dataset. Examples of how episodes of acute respiratory infection might be coded in GPRD are shown in table 4.3, along with whether the episode would be classified as ILI or general acute respiratory infection. Any ILI records were purged from the dataset, then remaining records were sorted and merged on patient identifier with AMI, demographic and registration records to generate a general acute respiratory infection dataset (dataset e).

Example episode	Example codes used	Date	Classification of episode
Episode 1	Influenza-like illness	06-12-2001	ILI
Episode 2	Acute bronchitis	27-02-2004	General ARI
Episode 3	Acute bronchitis Influenza-like illness	17-12-2003 19-12-2003	ILI
Episode 4	Influenza-like illness Acute bronchitis	21-05-2000 29-05-2000	ILI
Episode 5	Chest infection Streptococcal pneumonia	03-03-2007 05-03-2007	General ARI
Episode 6	Chest infection Influenza-like illness Streptococcal pneumonia	12-01-2002 13-01-2002 28-01-2002	ILI

Table 4.3 Example episodes with how they might be coded in GPRD and classification as an ILI or general ARI

4.4.9 Data management

For each of the five datasets (a= all acute respiratory infections including influenza, b= acute respiratory infections in weeks of influenza circulation, c= acute respiratory infections outside the influenza season, d= influenza-like illnesses only, e=general acute respiratory infections excluding ILI), infections

occurring outside the study period were dropped. Any AMI records in persons aged <40 years were removed as these persons were judged not to be clinically representative of the sample: triggers for AMI in those under 40 are likely to differ from those of older age groups eg hereditary disorders such as familial hypercholesterolaemia or recreational cocaine use may be more important. Dates of AMI and acute respiratory infection as well as the start and end date of follow up were converted to age in days at each of these events based on year of birth and an assumed birth date of 30th June for each individual (halfway through the calendar year). Data were then reshaped into a wide format in preparation for self-controlled case series analysis (see below).

Example of 'long' coding:
Id 1701 ARI date 07/05/2005 AMI date 03/06/2005
Id 1701 ARI date 02/12/2006 AMI date 03/06/2005

Example of 'wide' coding:
Id 1701 ARI date_1 07/05/2005 ARI date_2 02/12/2006 AMI date 03/06/2005

4.4.10 Statistical analysis

Weekly numbers of AMIs recorded in GPRD across the study period were presented graphically. Weekly numbers of GP consultations for acute respiratory illnesses and the subset of these coded as influenza-like illness were examined and compared with rates of ILI consultations per 100,000 population from RCGP surveillance data. Characteristics of participants including those with and without a record of acute respiratory infection were described.

Self-controlled case series analysis was performed to examine the incidence of AMI in time periods following a consultation for acute respiratory infection compared to baseline time periods for each person. The exposure period (during which it was hypothesised that an acute inflammatory stimulus such as influenza could plausibly provoke a systemic effect) was divided into 1-3, 4-7, 8-14, 15-28 and 29-91 days following an acute respiratory consultation. I excluded from baseline the time period from the day of consultation up to 14 days before as an

AMI occurring in this time window may affect subsequent likelihood of attending the GP (and self-controlled case requires that the probability of exposure is not affected by the occurrence of an outcome event). Incidence ratios were calculated for AMIs occurring in each exposure period compared to baseline time periods for each person in univariable analysis. I did not include fixed confounders such as gender and socio-economic status in models as self-controlled case series controls implicitly for such factors. I created multivariable models by adjusting for age in 1-year and then 5-year age-bands and for season in 3 month blocks (January – March, April – June, July – September, October – December) as these are time varying confounders associated with both AMI and risk of respiratory infection.

The initial analysis examined the risk of AMI occurring after acute respiratory infection classified using all general acute respiratory infection codes including influenza compared to other time periods (dataset a), adjusted for age and season. I also stratified by age-group and gender, testing for heterogeneity in incidence ratios for AMI occurring 1-3 days after respiratory consultation using the Cochran Q statistic. This is the classical measure of heterogeneity between studies in meta-analyses (calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies)[156] but it can also be used to measure heterogeneity in effect estimates across strata. It is known to have low power to test heterogeneity especially when the number of studies or strata is small[157].

Subsequent analyses examined AMI risks occurring after episodes of respiratory illness occurring in times of influenza circulation (dataset b) compared to those outside times of influenza circulation (dataset c) and compared the effect of episodes classified with influenza-like illness codes (dataset d) with those coded with general acute respiratory infection codes (dataset e) to explore whether any triggering effect was greater for illnesses judged more likely to be due to influenza. Again heterogeneity in incidence ratios for respiratory infection episodes judged more and less likely to be influenza was investigated using the Cochran Q statistic. Finally a sensitivity analysis was done to remove fatal episodes (where a patient had a record of death within 28 days of the AMI record)

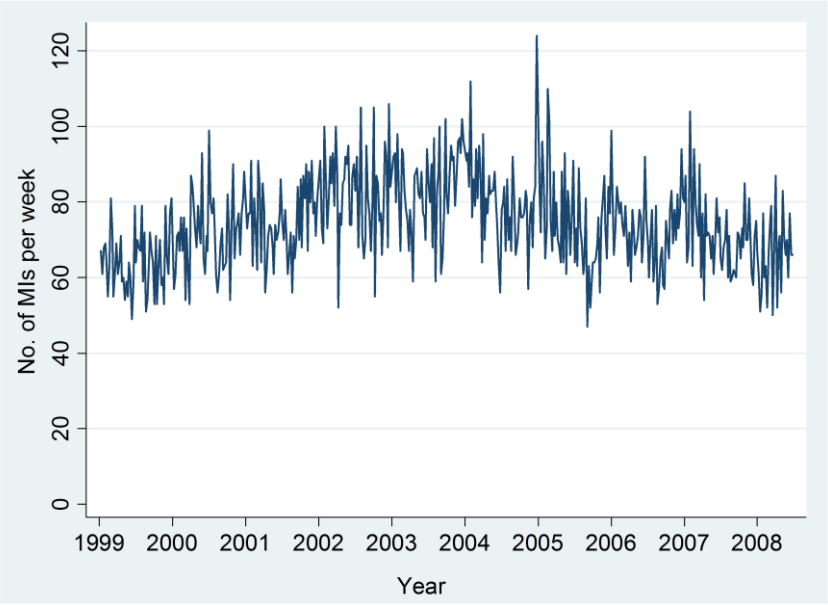
and compare results to those obtained in the initial analysis. All data management and analyses were done using Stata (*Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP).

4.5 Results

4.5.1 Description of outcome

From an original dataset of 38,274 records of first incident myocardial infarction occurring at least 6 months after patients had registered with the GP, 653 records were dropped for the following reasons: 40 occurred on the day of a routine health-check, 6 had a date of death preceding date of AMI, 607 occurred in people aged less than 40 years. The remaining 37,621 first AMIs that occurred in people aged ≥40 during the study period are shown in figure 4.2 below (mean = 3,726 per year). Incidence showed a seasonal pattern, with the graph peaking in winter months. Numbers of AMIs remained relatively stable across the study period, except for a dip at the end of 2008 (the final data collection period).

Figure 4.2 Number of AMIs recorded in GPRD per week 1999-2008

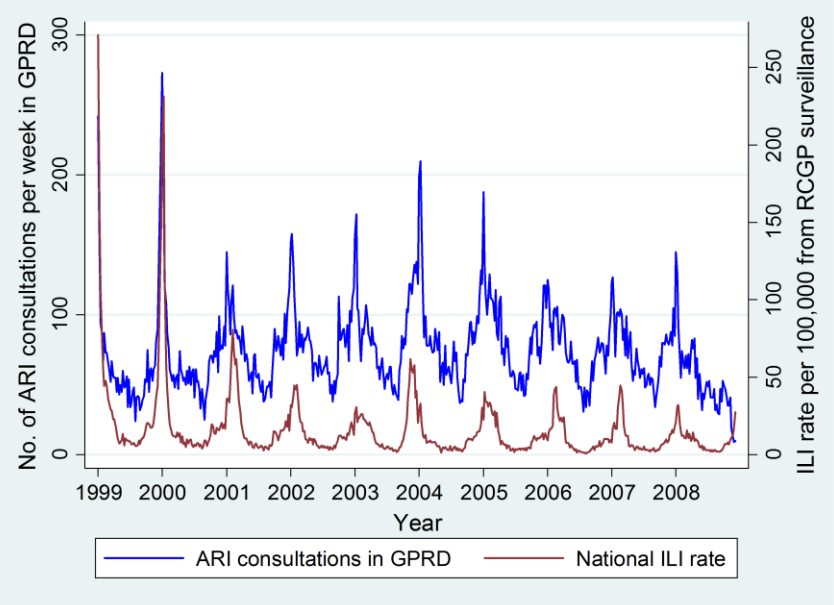


From this cohort of 37,621 patients with a first AMI, 15,917 also had a record of consultation for an acute respiratory infection so were eligible for inclusion in self-controlled case series analysis.

4.5.2 Description of exposure

15,917 patients had 38,519 records of GP consultations for acute respiratory infection during the study period (mean 2.4 among those with at least one record), of which 2,200 illnesses were classified as ILI. Acute respiratory infection consultations showed a very marked seasonal distribution, peaking in winter. Patterns of consultations for acute respiratory infection extracted from GPRD to form dataset a) are shown in figure 4.3 along with ILI rates from the RCGP surveillance scheme for the same time period. There was a very close match in temporal distribution of illnesses.

Figure 4.3 No. of ARI consultations recorded in GPRD per week from 1999-2008 compared to RCGP ILI rates per 100,000 from national surveillance schemes



4.5.3 Description of participants

Although only patients with a record of both incident AMI and acute respiratory infection were eligible for inclusion in the analysis, a comparison of characteristics of AMI patients with and without records of acute respiratory infection is shown in table 4.4. In general those without a record of acute respiratory infection during the study period were more likely to be male (64.7% versus 58.7%), had a slightly shorter period of follow up and were more likely to have died within 28 days of AMI. For the 15,917 AMI patients included in self-controlled case series analysis, 58.7% were male with a median age of 72.3 years

(IQR 61.9 - 80.7 years). Median follow up time was 9.0 years (IQR 6.2 - 10.0 years).

Characteristic	Patients with ARI record, n=15,917 No. (%)	Patients without ARI record, n=21,704 No. (%)
Gender		
Male	9,335 (58.7)	14,044 (64.7)
Female	6,582 (41.3)	7,660 (35.3)
Agegroup		
40-49	957 (6.0)	1,885 (8.7)
50-59	2,381 (15.0)	3,920 (18.0)
60-69	3,644 (22.9)	5,020 (23.1)
70-79	4,654 (29.2)	5,787 (26.7)
80-89	3,511 (22.1)	4,260 (19.6)
90+	770 (4.8)	832 (3.8)
Death within 28 days of AMI		
Y	1,963 (12.3)	3,853 (17.8)
N	13,954 (87.7)	17,851 (82.2)
Median follow up (years) (IQR)	9.0 (6.2-10.0)	8.4 (4.6-10.0)

Table 4.4 Characteristics of AMI patients with and without ARI records

4.5.4 Effect of general acute respiratory infection on AMI

Self-controlled case series analysis using dataset a) showed a marked increased risk of AMI occurring after GP consultation with acute respiratory infection – unadjusted incidence ratio 3.71(95% CI 3.15 - 4.38) for days 1-3, 2.99 (95% CI 2.54 - 3.51) for days 4-7, 2.53 (95% CI 2.21 - 2.89) for days 8-14, 1.89 (95% CI 1.69 - 2.11) for days 15-28 and 1.36 (95% CI 1.27 - 1.46) for days 29-91. Adjusting for age in 5 year age-bands gave similar results – IR 3.72 (95% CI 3.16 - 4.38) for days 1-3, 2.99 (95% CI 2.55 - 3.50) for days 4-7, 2.52 (95% CI 2.21 - 2.88) for days 8-14, 1.88 (95% CI 1.68 - 2.10) for days 15-28 and 1.30 (95% CI 1.22 - 1.39) for days 29-91.

In final multivariable models I adjusted for age in 5 year age-bands as well as season in 3-month blocks. This gave an adjusted incidence ratio of 3.65 (95% CI

3.10 - 4.30) for days 1-3, which tapered over time – see table 4.5. There were no significant differences in risks between men and women (p=0.62). In contrast risks increased markedly with age, from a small increased risk in those aged under 60 years –adjusted IR 1.81 (95% CI 1.05 - 3.13) – to a > five-fold increase in risk in the over 80's – adjusted IR 5.30 (95% CI 4.09 - 6.86) (p=0.001).

Model	Risk period after infection (days)	Age- and season-adjusted incidence ratio (95% CI)						
Overall	1-3	3.65 (3.10-4.30)	n=147±					
	4-7	2.93 (2.50-3.44)	n=156					
	8-14	2.47 (2.17-2.82)	n=228					
	15-28	1.84 (1.65-2.06)	n=332					
	29-91	1.28 (1.20-1.37)	n=1,067					
	Baseline	1.00	n=13,987					
		Men	Women	p∞				
By gender	1-3	3.53 (2.83-4.40)	3.83 (3.00-4.90)	0.62				
	4-7	2.93 (2.37-3.61)	2.95 (2.32-3.76)					
	8-14	2.62 (2.21-3.10)	2.30 (1.86-2.84)					
	15-28	1.83 (1.58-2.12)	1.87 (1.58-2.22)					
	29-91	1.15 (1.04-1.26)	1.47 (1.33-1.63)					
	Baseline	1.00	1.00					
		<60 years	60-70 years	p	70-80 years	p	>80 years	p
By age group*	1-3	1.81 (1.05-3.13)	3.27 (2.29-4.68)	0.076	3.38 (2.50-4.58)	0.051	5.30 (4.09-6.86)	0.001
	4-7	2.83 (1.93-4.14)	2.08 (1.41-3.06)		2.44 (1.79-3.33)		4.20 (3.25-5.41)	
	8-14	1.74 (1.20-2.51)	1.75 (1.26-2.42)		2.75 (2.19-3.44)		3.20 (2.56-4.00)	
	15-28	1.27 (0.94-1.73)	1.50 (1.17-1.93)		2.09 (1.73-2.52)		2.19 (1.80-2.66)	
	29-91	1.05 (0.88-1.24)	1.05 (0.90-1.23)		1.28 (1.13-1.45)		1.60 (1.41-1.80)	
	Baseline	1.00	1.00		1.00		1.00	

Table 4.5 Adjusted IRs for AMI after ARI overall and stratified by gender and age group

±Number of AMI events occurring in each risk period

∞p values presented from Cochran Q test for heterogeneity in incidence ratios for AMI in days 1-3 after acute respiratory infection between different strata.

*For each age group, incidence ratios for AMI in days 1-3 after acute respiratory infection are compared with the result for <60 years

4.5.5 Comparison of effects of ARI episodes judged more and less likely to be due to influenza on AMI

Results from self-controlled case series using dataset b) (episodes of acute respiratory infection occurring in influenza circulation weeks) compared to dataset c) (episodes occurring outside of times of influenza circulation) are shown in table 4.6 below. Episodes occurring during peak weeks of influenza circulation did not have a significantly greater effect on AMI than episodes occurring at other times, using definitions 1, 2 or 3 for the influenza season. Episodes coded with an ILI code had a slightly higher adjusted incidence ratio than those with a general respiratory infection code but this difference was not statistically significant. For this analysis IRs remained high (>3) up to day 14 following consultation with an acute respiratory infection.

Risk period after infection (days)	Age- and season-adjusted incidence ratio (95% CI)		
	Peak weeks (definition 1)	Non peak weeks (definition 1)	P value
1-3	2.91 (1.85-4.59)	3.66 (3.07-4.36)	0.36
4-7	3.49 (2.43-5.03)	2.75 (2.30-3.27)	
8-14	2.44 (1.74-3.40)	2.38 (2.06-2.75)	
15-28	1.64 (1.23-2.20)	1.78 (1.58-2.01)	
29-91	1.25 (1.05-1.48)	1.26 (1.17-1.36)	
Baseline	1.00	1.00	
	Peak weeks (definition 2)	Non peak weeks (definition 2)	P value
1-3	2.84 (1.93-4.19)	3.72 (3.10-4.45)	0.22
4-7	2.98 (2.14-4.15)	2.81 (2.35-3.37)	
8-14	2.16 (1.60-2.90)	2.44 (2.11-2.83)	
15-28	1.67 (1.31-2.13)	1.77 (1.56-2.01)	
29-91	1.12 (0.96-1.30)	1.28 (1.19-1.38)	
Baseline	1.00	1.00	
	Peak weeks (definition 3)	Non peak weeks (definition 3)	P value
1-3	3.25 (2.34-4.50)	3.62 (2.99-4.37)	0.57
4-7	2.66 (1.94-3.64)	2.91 (2.42-3.49)	
8-14	2.19 (1.68-2.85)	2.44 (2.09-2.84)	
15-28	1.58 (1.26-1.98)	1.80 (1.59-2.05)	
29-91	1.18 (1.04-1.35)	1.27 (1.18-1.38)	
Baseline	1.00	1.00	
	ILI code	General ARI code	P value
1-3	3.87 (2.00-7.46)	3.56 (3.00-4.22)	0.81
4-7	3.26 (1.75-6.08)	2.89 (2.45-3.41)	
8-14	3.61 (2.29-5.69)	2.35 (2.05-2.71)	
15-28	1.45 (0.87-2.41)	1.86 (1.66-2.08)	
29-91	1.31 (1.01-1.70)	1.28 (1.20-1.38)	
Baseline	1.00	1.00	

Table 4.6 Adjusted IRs for AMI after ARI episodes judged more and less likely to be due to influenza

Definition 1 = 2 weeks either side of peak week, definition 2 = 3 weeks either side of peak week, definition 3 = 4 weeks either side of peak week

4.5.6 Sensitivity analysis excluding fatal events

Of 15,917 AMI events included in the above analyses, 1,963 (12.3%) had an earliest record of death that was within 28 days of AMI date, of which 1,675 (10.5%) deaths occurred on the day of AMI. When these records were excluded from self-controlled case series the adjusted IR for AMI fell to 2.49 (95% CI 2.01 - 3.09) for days 1-3, IR 2.23 (95% CI 1.83 - 2.72) for days 4-7, IR 2.06 (95% CI 1.76 - 2.41) for days 8-14, IR 1.50 (95% CI 1.32 - 1.72) for days 15-28 and IR 1.13 (95% CI 1.05 - 1.22) for days 29-91.

4.6 Discussion

4.6.1 Summary of findings

In summary there was a 3.65 fold increase in the age- and season-adjusted risk of AMI in the first three days following GP consultation with acute respiratory infection, which tapered over time. Risks were highest in those aged over 80 years. There were no significant differences in risks of AMI associated with episodes of acute respiratory infection occurring in time periods when influenza was circulating compared to other time periods, or for episodes classified using influenza-like illness codes rather than general acute respiratory infection codes. Risks were diminished to around 2.5 fold when fatal events were excluded.

4.6.2 Strengths of study

This study was conducted in a large representative sample of the UK population so results are likely to be generalisable to adults in the UK. Quality of GPRD data is high and the database has been well validated with a median of 89% of GPRD-recorded diagnoses confirmed for all cases through both internal and external sources of validation[149]. Self-controlled case series analysis has the major advantages of reducing risks of bias through inappropriate choice of controls (as people act as their own controls) and implicitly controlling for fixed confounders[150]. This is particularly important when using routinely collected primary care data, where there may be variable recording of potential

confounding factors. Another advantage of self-controlled case series is the ability to use multiple risk periods, which allowed us to identify a gradient of risk for AMI after acute respiratory infection. We did not know the onset date of infections but instead used the date of diagnosis. In one study of ILI in general practice 80% of patients consulted more than 36 hours after symptom onset[158] while another study of sore throat showed that one third of patients had symptoms of >3 days duration[159]. As the date of attending the GP is likely to be some time after the onset of respiratory symptoms, the true effect on AMI may be even higher than that observed.

4.6.3 Study limitations - timing of AMI

Using the GPRD to obtain information on AMI – a condition where care would have been received in hospital rather than at the GP – introduces the potential for inaccurate recording. While the diagnosis itself is likely to be accurate[149], potential issues arise with regard to sensitivity and timing. Missed recording of AMI in GPRD (ie false negatives) would not introduce bias into the analysis; the study power would simply be reduced by a smaller sample size. Inaccurate data entry on timing of the event is potentially more problematic: for self-controlled case series analysis using short risk periods, even a small proportion of AMI records with inaccurate dates could introduce considerable bias. While I removed records of AMI events recorded on the same day as routine health checks or within 6 months of a patient registering with a practice, this only removed a relatively small number of records. With no way to validate timing of AMI events, the use of influenza surveillance data to define the influenza season is rendered less accurate as it relies heavily on fairly tightly defined time windows of virus circulation. Some factors in the dataset and results suggest that there is a residual risk of retrospective recording of AMI. First it is known that death records have a median 19 days delay from true date of death, and AMI records are entered in a similar way. Second there was a peak in AMI records for the 1st January each year that suggests recording error. Third, high incidence ratios for AMI after respiratory infection were apparent for several weeks (whereas the likely biological effect would occur days after infection, suggesting delayed recording of AMI. This was particularly apparent for ILI episodes where the

incidence ratio remained >3 for up to 14 days. If AMI recording were delayed, this would suggest that the true effect of acute respiratory infections including ILI on AMI was higher than that observed.

4.6.4 Study limitations - diagnosing influenza using primary care data

Identifying influenza infections in primary care data is challenging as cases rarely have microbiological confirmation. While some studies have based influenza diagnosis on codes used by GPs to classify respiratory illnesses[160,161], this method alone would fail to identify influenza infections classified using non-specific respiratory illness codes. In this study relatively few illnesses were classified with specific ILI codes. This decreased statistical power to detect an effect in the 'ILI' group and diluted the 'non-ILI' group; while point estimates varied substantially, the difference between groups was not significant. I also identified illnesses likely to be caused by influenza through linked influenza surveillance data because the positive predictive value for influenza of having an acute respiratory infection during time periods when influenza is circulating is known to be high[34]. No greater effect was observed in peak weeks of influenza circulation compared to non-peak weeks, however, despite varying the number of weeks included in the 'peak' and 'non-peak' periods. This would suggest that either influenza has a similar effect compared to other respiratory infections or alternatively that this method was insufficiently sensitive to divide illnesses into influenza and non- influenza episodes. As described above, accurate timing of AMI events is crucial for this stratified analysis. Other possible approaches to addressing this question are discussed in section 4.6.6 (future directions).

4.6.5 Interpretation of results in context of previous findings

Previous large primary care database studies using either case control or case only designs have shown an association between GP attendance with acute respiratory infection and subsequent AMI. My incidence ratio for AMI in days 1-3 after acute respiratory infection – IR 3.65 (95% CI 3.10 - 4.30) – was in the same direction as but somewhat lower than that of an earlier comparable self-controlled case series study in GPRD – IR 4.95 (95% CI 4.43 - 5.53)[84]. Though I

adjusted for season as a proxy for environmental factors which would tend to lead to a more conservative effect size, this would not account for all of the difference in incidence ratios. The time period of the study and codelists used to extract incident myocardial infarction and acute respiratory infection records were also different, which may account for the different effect size seen. I identified a biological gradient of effect on AMI which was also apparent after acute respiratory infection in this other self-controlled case series analysis[84] and in two other primary care database studies using a case control design[80,83]. As with our study, previous individual level studies attempting to focus on a specific triggering effect of influenza have shown mixed results. One Chinese study in a largely unvaccinated population found a significant association between the presence of serum influenza antibodies and AMI[91] but three other small case control studies were underpowered and failed to detect any association[87–89]. Whether influenza is more likely than other acute respiratory infections to trigger AMI remains an area for further study.

4.6.6 Future directions

Better data are needed on the timing of AMI events, as well as on diagnosis to ensure that AMIs recorded meet standard international criteria[58]. This would also allow stratification by AMI type which is important because different pathophysiological pathways underlying ST- and non ST-segment elevation myocardial infarctions (STEMI and NSTEMI) might be more or less susceptible to interaction with or triggering effects of respiratory viruses. Better data on AMI timing would also help to ensure the validity of analyses based on timing of the influenza season. In chapter 5 similar analyses are performed in linked datasets to address some of the study limitations.

SUMMARY

- A self-controlled case series analysis was carried out using General Practice Research Database records to examine the incidence of AMI in time periods following GP consultation with acute respiratory infection compared to other time periods.
- 37,621 people aged ≥40 years had a first AMI during the study period (1999-2008), of whom 15,917 had also consulted for acute respiratory infection.
- There was a 3.65-fold (95% CI 3.10 - 4.30) increase in the age- and season-adjusted risk of AMI in the first three days after acute respiratory infection, which fell to baseline by 28 days
- Risks were greatest in people aged over 80 years but no gender differences were seen.
- There was no effect of stratifying ARI episodes by levels of circulating influenza or codes used to classify illness, although whether influenza is more likely to trigger AMI than other acute respiratory infections remains inconclusive.

5. Extension to self-controlled case series analysis using linked databases: GPRD, MINAP and HES

5.1 Description of chapter contents

This study extends work carried out in the previous chapter by using primary care data on acute respiratory infections from the General Practice Research Database (GPRD) linked to detailed information on diagnosis and timing of AMI through the Myocardial Ischaemia National Audit Project (MINAP) database and Hospital Episode Statistics (HES). MINAP is a cardiac disease registry designed to capture in-depth information on all episodes of acute coronary syndrome presenting to hospitals in England & Wales. Approximately half of GPRD practices are linked to MINAP. HES is a national data warehouse for England containing details of all admissions to NHS hospitals, with reason for admission classified by International Classification of Diseases (ICD) code. Individually-linked anonymised patient records from GPRD, MINAP and HES were brought together by the CArdiovascular disease research using Linked Bespoke studies and Electronic Records (CALIBER) programme.

In this chapter, self-controlled case series analysis is used to examine the association between GP consultation for acute respiratory infection and incident myocardial infarction. A comparison of results when using AMI recorded in MINAP, HES and GPRD is done, with models adjusted for age and season. Effects are examined by age, gender, type of AMI and presence of previous vascular disease. A number of sensitivity analyses are performed. Subsequent models examine whether episodes of acute respiratory infection judged more likely to be caused by influenza (through medical codes used to classify illness, timing of the episode in relation to the influenza season and vaccination status of the patient at the time of infection) are associated with higher risks of AMI than other episodes.

5.2 Study rationale and introduction to data sources

Use of linked datasets allows collation of information from sources including primary and secondary care and disease registries to enhance the breadth of data available for research. For this study, linked data were used to ensure that the most accurate and complete data were available on timing of AMI, which is essential for self-controlled case series analysis. The data linkage also allowed the AMI diagnosis to be refined, for example by investigating whether influenza was more likely to trigger STEMIs than NSTEMIs, which would not be possible using GPRD data alone. In general linked datasets also offer the opportunity to validate outcomes, although this was not done formally here.

The CArdiovascular disease research using Linked Bespoke studies and Electronic Records (CALIBER) programme[162] links information from GPRD and MINAP using patient pseudo-identifiers and an encrypted key file. Pseudo-identifiers are replaced with a CALIBER identifier to produce a fully anonymised dataset for use in research. Currently linked data are available for the time period 01/01/2003 until 31/07/2009.

5.2.1 Myocardial Ischaemia National Audit Project (MINAP)

MINAP contains anonymised electronic records of hospital admissions for acute coronary syndromes from all hospitals in England & Wales where such patients are admitted[163]. MINAP began in 1998 with the aim of developing a dataset of acute STEMIs to allow clinicians to evaluate their management of patients against national standards specified by the National Service Framework for Coronary Heart Disease[164]. Subsequently the dataset expanded to include all acute coronary syndromes. In 2011 the dataset contained records on 873,000 patients[165]. These records contain information on demographic details, medical history, date and timing of episode, diagnosis, investigations and treatment. Further detail on the MINAP dataset structure relevant to this analysis is given in section 5.4.1.

Quality is monitored continuously through assessment of the completeness of 20 key data entry fields for patient with NSTEMI. Nationally these fields are 99% complete[165]. In addition there is an annual data validation study in which hospitals are required to re-enter data from 20 randomly selected case records in 20 fields into an online data validation tool. Data are compared between the original entry and the re-entered data for each variable. In 2011 97.5% of eligible hospitals in England and Wales participated in the data validation study and the median hospital score was 94.8% (IQR 90.0 - 97.8)[165].

5.2.2 Hospital Episodes Statistics (HES)

HES is a large secure national data warehouse for England containing details of all admissions to NHS hospitals. Data on episodes of admitted patient care are available from 1989 onwards, with more than 12 million records added each year[166]. Each 'episode' of care under a different consultant has a new record, so one patient may be responsible for several episodes within the dataset, even within the same hospital admission. The ICD coding system[167] is used to record reasons for admission, with the ICD-10 classification in use since 1995. Types of information contained in HES records includes clinical data on diagnosis and procedures undergone, demographic details such as age, gender and ethnicity, administrative information such as date of admission and geographical details such as area of residence. Further details on variables used from the HES dataset in this analysis are given in section 5.4.1.

The HES Data Quality team checks and reports quality of data during processing based on factors such as the level of population of all fields, use of obsolete or invalid provider codes, numbers of potential duplicate episodes and comparisons of monthly activity compared to previous months. A monthly quality report is available at HES online as well as a high level summary of key figures.

5.2.3 General Practice Research Database (GPRD)

The GPRD has been described in detail in chapter 4 (sections 4.2.1 and 4.4.1). GPRD records used for this analysis were slightly different as they were provided

by CALIBER and contained a CALIBER identifier rather than a GPRD identifier. Individual CALIBER datasets given to researchers do not contain exhaustive information on all variables found in GPRD but instead are tailored to the individual project. This dataset contained records on registration, demographics, cardiovascular risk factors, AMI, episodes of acute respiratory infection, influenza vaccinations and deaths. Episodes of acute respiratory infections and influenza vaccinations were extracted using updated codelists to reflect the new GPRD Gold classification system.

5.3 Aims and objectives

Aim: To investigate the association between episodes of acute respiratory infection, in particular those likely to be caused by influenza, and AMI using linked data from GPRD, MINAP and HES.

Objectives:

1) To examine whether there was a greater incidence of AMI recorded in MINAP (primary outcome) in time periods following GP consultation for acute respiratory infection using self-controlled case series adjusted for age and season.
2) To compare the above with results obtained using the secondary outcomes AMI recorded in HES and AMI recorded in GPRD.
3) To investigate whether any effect varied by age, gender, type of AMI and history of vascular disease.
4) To explore whether acute respiratory infections that a) occurred during time periods when influenza was circulating, b) were coded with ILI codes, c) occurred in unvaccinated individuals or d) had a combination of the above indicators of influenza were more likely to be associated with AMI than other illness episodes.
5) To test the robustness of effect estimates through various sensitivity analyses including a) exclusion of fatal AMI episodes, b) exclusion of episodes where symptom date was inferred from hospital discharge date, c) inclusion of records with missing discharge diagnosis, d) adjusting for age in 1 year bands.

5.4 Methods

5.4.1 Data sources

For these analyses, MINAP records and then separately HES records and GPRD records were used as the source of information on outcome while, as before, GPRD records were used to obtain information on exposure and follow up. MINAP data are collected directly from hospital medical records by nurses and clinical audit staff and entered securely via a dedicated data application[165]. Information collected includes demographic details, the presence or absence of cardiovascular risk factors, previous cardiac drug use, timing of the AMI episode (including separate data entry fields for date and time of: symptom onset, call for help, arrival of help, arrival of services, admission to hospital etc), diagnosis on admission and discharge eg STEMI or NSTEMI, procedures performed such as angiography and treatments received. HES records contained an anonymous patient identifier for linkage to GPRD and MINAP, admission and discharge dates of the hospitalised episode as well as ICD codes used to classify AMI. Further detail on outcome definitions is given in section 5.4.5.

5.4.2 Study population and follow up

Potentially eligible patients were those registered with a GPRD practice linked to MINAP during the study period 01/01/2003 until 31/07/2009 and who had a first AMI recorded in MINAP aged ≥40 years. As for the previous study, follow up began either at the date of registration with a GPRD practice or at the practice's 'up-to-standard' date or at the study start date (01/01/2003), whichever was latest. Follow up ended either at the study end date (31/07/2009) or at the date of censoring (due to death or leaving the practice), whichever was earlier. Dates of death were obtained from a file provided by CALIBER containing the CALIBER patient identifier and death dates (originally from GPRD). As before if there were discrepancies the earliest date of death was used. This was used as the end date of follow up unless it was more than 95 days before the leaving date of the

practice and the leave reason was 'death', in which case the date of end of registration was used (according to advice in GPRD documentation).

5.4.3 Sample size calculations

As in chapter 4, the user written Stata command 'sampsi_sccs' was used to calculate sample size requirements. For an incidence ratio ('rho') of 3, a duration of the post exposure risk period of 28 days and a duration of the entire observation period of 2,403 days (based on median length of follow up) the sample size needed would be 453 to estimate results with 90% power at the 5% significance level. Table 5.1 below shows the different sample sizes required for this study under varying conditions.

Post exposure risk period (days)	Rho = 3	Rho = 2
91	144	436
28	453	1345
14	856	2658
7	1697	5286

Table 5.1 Sample sizes needed for self-controlled case series in MINAP under different conditions

5.4.4 Explanation of codelists used

For this analysis, codelists for outcome and exposure events were obtained in several different ways depending on the origin of required variables. For the outcome AMI, the codes used to extract records were either MINAP discharge diagnosis codes, HES ICD-10 codes or GPRD Gold codes (with a codelist generated by the CALIBER team) depending on the dataset used to generate AMI records. For the exposure acute respiratory infection (later subdivided into ILI and other codes according to table 4.2) GPRD Gold codelists of Read codes were used to extract records. These were generated by Dr Sara Thomas at LSHTM, a collaborator on this work, using similar methods to those described previously. Dr Thomas also provided GPRD Gold codelists for influenza vaccination, which were used to divide records of acute respiratory infection into those more and less likely to be due to influenza infection depending on an individual's vaccination status. Further details of outcome codes are given in section 5.4.5

and exposure codes in section 5.4.6. For all GPRD-derived variables, records were extracted by the CALIBER team. AMI records and acute respiratory infection records came from GPRD Clinical files; influenza vaccination records were extracted from GPRD Immunisation and Therapy records. Datasets containing a unique CALIBER patient identifier, medical code and date of event were provided by CALIBER for use in this analysis.

5.4.5 Outcomes

5.4.5.i Acute myocardial infarction recorded in MINAP (primary outcome)

Identification of AMI events was based on codes entered in the discharge diagnosis field (see appendix 10.1.2). Records were deleted if they contained the following codes that were unlikely to relate to AMI, such as 'Chest pain ?cause', 'Acute coronary syndrome – troponin negative', 'Other', 'Threatened myocardial infarction', 'Myocardial infarction unconfirmed' and 'Missing'. Records were also deleted where the discharge diagnosis was 'Acute coronary syndrome –troponin unconfirmed' if there was no separate confirmation of a positive cardiac marker result. All remaining records met internationally agreed criteria for AMI[58] and could be further subdivided into STEMI and NSTEMI based on troponin and ECG findings following the universal definition. In this study there was no need to restrict to AMI events that happened at least 6 months after a patient registered with a practice because using the MINAP-recorded outcome removed the risk of retrospective recording. As in the previous study only first events were included. Therefore after removing records of subsequent AMI events in MINAP, the MINAP dataset was merged with the linked GPRD dataset of myocardial infarction records. MINAP records of AMI were deleted if there was both a positive history of AMI noted in the MINAP record *and* a confirmatory AMI code in the GPRD record occurring more than 28 days before the MINAP recorded event.

When no symptom onset date was recorded in MINAP (31.5% of records), I substituted the date of call for help (11.6%), arrival of help (0.2%), arrival of services (0.8%), admission (16.1%), reperfusion (0.06%), cardiac arrest (0.05%), referral for investigation (0.6%), local angiogram (0.2%) or first local intervention (0.03%). The median number of days from symptom onset to

hospital discharge was calculated and used to estimate symptom onset date for a small number of records where only the date of discharge was available (1.5%). Records with no dates attached were excluded (0.4%). This dataset of first AMI events recorded in MINAP was then merged with demographic and registration information for each individual from GPRD and date checks done to ensure that no AMI records occurred before the start or after the end of a person's follow up.

5.4.5.ii Acute myocardial infarction recorded in HES (secondary outcome)

A series of linked HES records of AMI coded by ICD-10 diagnosis were used as a secondary outcome in sensitivity analysis. Records containing ICD-10 codes I21 (acute myocardial infarction) and I23 (complications following acute myocardial infarction) were retained and merged with patient demographic and registration information from GPRD. Records containing ICD-10 codes I22 (subsequent myocardial infarction) were excluded as these were, by definition, not first AMI events. The admission date was used as a proxy for event date. Again I did not specify a minimum length of follow up needed in GPRD prior to AMI as recording AMI events separately (in HES) should minimise the risk of retrospective recording. AMI records occurring before the start or after the end of a person's follow up in GPRD were removed. I excluded records relating to hospitalisations for second or subsequent infarctions in HES. Records were also excluded where there was a previous AMI recorded in the linked GPRD record occurring at least 28 days before the admission date in HES. It was not possible to subdivide records further into type of AMI as ICD-10 codes do not allow easy distinction between STEMI and NSTEMI. This dataset of first AMI events recorded in HES was then merged with demographic and registration information for each individual from GPRD. Any AMI records occurring before the start or after the end of a person's follow up were excluded.

5.4.5.iii Acute myocardial infarction recorded in GPRD (secondary outcome)

AMI recorded in GPRD was used as another secondary outcome in this analysis. CALIBER provided three datasets of AMI records extracted from GPRD for STEMI, NSTEMI and myocardial infarction with phenotype not otherwise specified. <10% of GPRD AMI records had a specified phenotype so all codes were combined to give a general 'AMI' outcome. The AMI date entered was the event

date specified by the hospital in the discharge letter sent to the general practice. Records of AMI from the three datasets were appended and the earliest record for each person was taken as the date of first event. Records of events occurring less than 6 months after the start of a patient's registration with a practice were removed. As only specific GPRD records related to outcome, exposures and follow up were extracted by CALIBER, events recorded on the same day as a routine health check or new patient screen were unable to be removed. As before, AMI records were merged with demographic and registration information for each individual. When using the GPRD AMI outcome, unlike the MINAP or HES outcomes, both events and follow up time were generated from the same dataset so it was unlikely that AMI events would be recorded outside a patient's period of registration with the GP. Follow up time for each individual was also constrained by the dates that linked data were available (01/01/2003 – 31/07/2009) and some patients had historical records dating back decades. Therefore any AMI records occurring outside of a person's follow up were removed.

5.4.6 Exposure

As for analyses in the previous chapter, data on primary care consultations for acute respiratory infection were extracted from GPRD Clinical files using four codelists. The main codelist (codelist 1) contained codes to describe acute respiratory infection with a systemic component eg 'acute bronchitis', 'tracheitis', 'pneumonia' designed to capture more severe episodes of acute respiratory infection presenting to general practice. Any organism-specific codes that did not relate to influenza (eg staphylococcal pneumonia) were then excluded to define a subset of codes where the diagnosis could plausibly be influenza (codelist 2). Finally I divided codes from the main list into two further subsets: one contained specific ILI codes only (codelist 3) and the other contained all other codes for comparison (codelist 4). Any consultations for acute respiratory infection occurring within 28 days of a previous consultation were excluded as they were likely to relate to the same illness. ARI codelists are given in appendix 10.1.3.

5.4.7 Identifying 'influenza' infections

Identifying acute respiratory infections judged to be more and less likely to be caused by influenza was done in three ways. First (as before – see section 4.4.8) the effect on AMI of episodes coded with an ILI code was compared to the effect of episodes coded with a general acute respiratory infection code (list 3 versus list 4). Second, levels of circulating influenza according to surveillance data were used to define peak time periods of influenza circulation (see section 4.4.7). Episodes of acute respiratory infection that could plausibly be due to influenza were extracted using list 2 and classified as occurring at either peak or non-peak times of influenza circulation. Third I categorised the influenza vaccination status of patients at the time of acute respiratory infection as either 'vaccinated' (making an infection less likely to be caused by influenza virus), having 'residual protection' or as 'unvaccinated' (when acute respiratory infections were most likely to be caused by influenza). 'Vaccinated' episodes occurred when influenza vaccination had been received at least 14 days before the illness in the same year (classed as 1st September until 31st August to encompass the start of the influenza vaccination season). Episodes with 'residual protection' were those in which a patient had received influenza vaccination in the last 5 years but not in the same year in which the infection occurred. 'Unvaccinated' episodes occurred when there had been no influenza vaccination in the last 5 years. For this analysis episodes of acute respiratory infection were also extracted with codes from list 2 to maximise sensitivity. Finally I examined the effect of illness episodes with increasing numbers of indicators of influenza infection: illness during influenza circulation weeks, ILI code and unvaccinated status.

5.4.8 Data management and statistical analysis

Any records of acute respiratory infection occurring outside a person's follow up period were dropped. Records of AMI occurring in persons aged <40 years were also removed from the dataset. Dates of AMI and acute respiratory infection as well as the start and end date of follow up were converted to age in days at each of these events. For analyses using the MINAP AMI outcome I was able to obtain a more exact date of birth than in the previous chapter using information from the

MINAP dataset in which age in years at admission was recorded to two decimal places. This was converted to age in days at event then subtracted from the admission date to give a birth date. For the HES and GPRD AMI outcomes, records of birth year were obtained from GPRD and date of birth calculated by assuming a birth day of 30th June (halfway through the calendar year). Data were reshaped into a wide format in preparation for self-controlled case series analysis.

A flowchart of records was generated to illustrate when and why potential AMI records were dropped. Characteristics of participants with a record of acute respiratory infection were described. Self-controlled case series analysis was used to examine the incidence of MINAP-recorded AMI (the primary outcome) in time periods following a consultation for acute respiratory infection compared to baseline time periods for each person. As before the exposure period was divided into 1-3, 4-7, 8-14, 15-28 and 29-91 days following an acute respiratory consultation. The time period from the day of consultation up to 14 days before was excluded from baseline as an AMI occurring in this time window may affect subsequent likelihood of attending the GP. Incidence ratios were calculated for AMIs occurring in each exposure period compared to baseline time periods for each person adjusted for age in 5-year agebands and season in 3-month blocks.

The initial analysis examined the risk of AMI occurring after any acute respiratory infection classified using list 1 compared to other time periods. I stratified by age-group, gender, type of AMI (STEMI v. NSTEMI) and history of previous vascular disease (defined by a history of angina, percutaneous coronary intervention, coronary artery bypass graft, peripheral vascular disease or cerebrovascular disease recorded in MINAP) and tested for heterogeneity using the Cochran Q statistic. Results obtained from this analysis were compared with results when using HES AMI and then GPRD AMI as an outcome. I carried out several sensitivity analyses including 1) adjusting for age in 1 year agebands, 2) excluding records where the patient died in hospital, 3) excluding records for which symptom onset date was inferred using discharge date and average length of stay and 4) including records with missing discharge diagnoses.

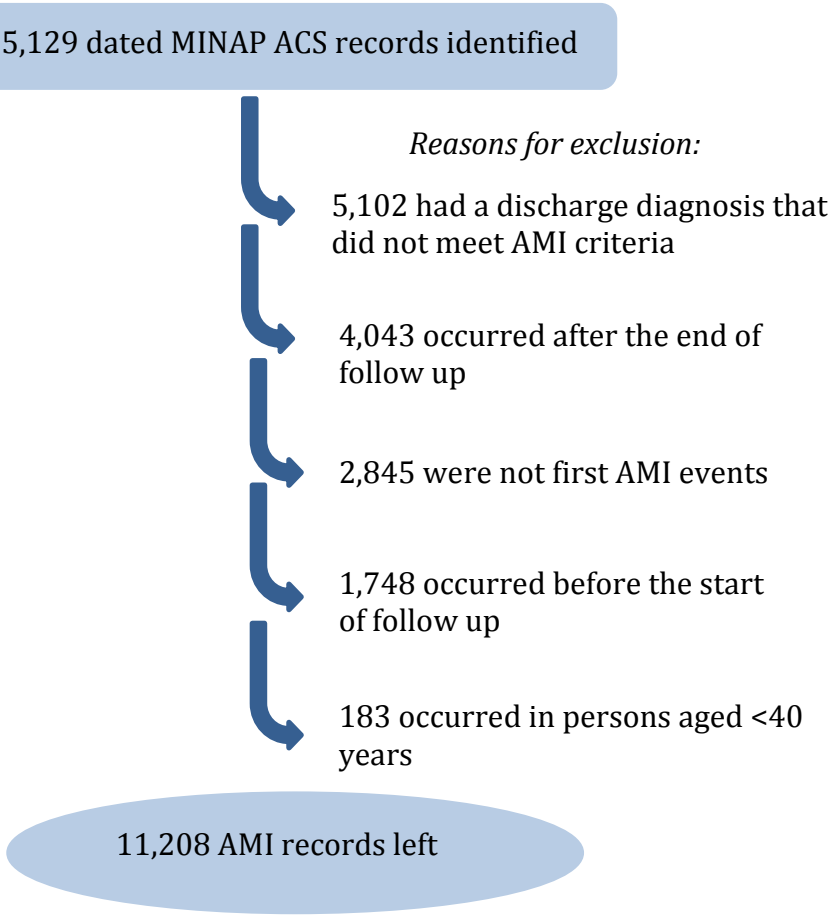
Subsequent analyses examined AMI risks occurring after episodes of respiratory illness most likely to be caused by influenza to explore whether any triggering effect was specific to influenza. These analyses used the primary outcome MINAP-recorded AMI. I compared the effect on AMI of 1) influenza-like illnesses with general acute respiratory infections, 2) acute respiratory infections at times of peak influenza circulation with illnesses at non-peak times, 3) acute respiratory infections after influenza vaccination with illnesses occurring in unvaccinated time periods or time periods with residual protection, and 4) acute respiratory infections with increasing numbers of markers of influenza with illnesses with no markers of influenza. Again heterogeneity in incidence ratios for respiratory infection episodes judged more and less likely to be influenza was investigated using the Cochran Q statistic. All analyses were done using Stata (*Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP).

5.5 Results

5.5.1 Description of primary outcome

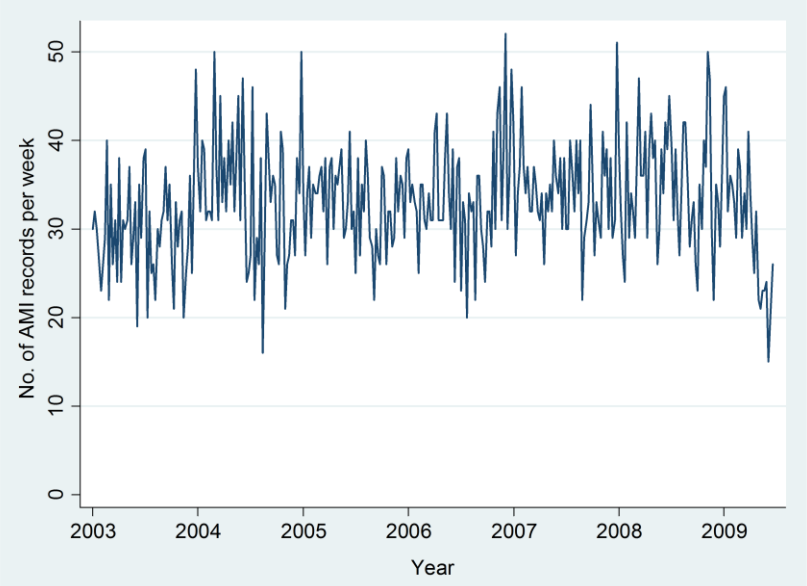
25,129 potential myocardial infarction records were identified in the MINAP database for 22,024 persons. Of these 13,921 records were dropped for reasons outlined in figure 5.1.

Figure 5.1 Flowchart of AMI records identified in MINAP dataset. Note that some records could have been excluded for multiple reasons but records were excluded according to the hierarchy shown



The weekly pattern of first AMIs recorded in MINAP during the study period is shown in figure 5.2. Numbers remain relatively stable over time and there is some evidence of a seasonal pattern with numbers peaking in winter.

Figure 5.2 Number of AMIs recorded in MINAP per week from 2003-2009



5.5.2 Description of participants

Of the remaining 11,208 persons with a first AMI record, 3,927 (35.0%) had also consulted for an acute respiratory infection and were included in analyses. These individuals had 8,204 episodes of acute respiratory infection (mean 2.1 per person) during the study period. Their median age was 73.1 years (IQR 62.5 - 81.4) and 60% were male (table 5.2).

Characteristic	No. (%)
Gender	
Men	2,360 (60.1)
Women	1,567 (39.9)
Age group	
40–49	212 (5.4)
50–59	565 (14.4)
60–69	873 (22.2)
70–79	1,122 (28.6)
80–89	945 (24.1)
≥90	210 (5.3)
History of vascular disease†	
Angina	839 (25.9)
PCI∞	130 (4.1)
CABGα	124 (3.9)
Peripheral vascular disease	144 (4.7)
Cerebrovascular disease	282 (9.1)
Type of myocardial infarction	
STEMI	1,604 (40.8)
NSTEMI	2,323 (59.2)
Death in hospital†	
Y	250 (6.6)
N	3,548 (93.4)

Table 5.2 Characteristics of study participants (n=3,927)

†% given out of those with non-missing data
∞PCI = percutaneous coronary intervention; αCABG = coronary artery bypass graft

5.5.3 Effect of general acute respiratory infection on primary outcome (MINAP-recorded AMI)

Risk of AMI was substantially higher in days following acute respiratory infection – adjusted IR 4.19 (95% CI 3.18 - 5.53) for days 1–3 – with the effect tapering over time. The effect was most marked in the oldest age groups, with the adjusted IR rising to 5.94 (95% CI 3.90 - 9.04) for days 1–3 after acute respiratory infection in the over 80s (p=0.023). There was no evidence of interaction by gender (p=0.82), type of AMI (p=0.53) or history of previous vascular disease (p=0.73)(table 5.3).

Model	Risk period after infection (days)	Age– and season–adjusted incidence ratio (95% CI)						
Overall	1-3	4.19 (3.18–5.53)	n=52[±]					
	4-7	2.69 (1.99–3.63)	n=44					
	8-14	1.66 (1.24–2.23)	n=48					
	15-28	1.41 (1.12–1.77)	n=80					
	29-91	1.05 (0.92–1.21)	n=262					
	Baseline	1.00	n=3441					
		Men	Women	p[∞]				
By gender	1-3	4.32 (3.02–6.19)	4.05 (2.62–6.27)	0.82				
	4-7	2.01 (1.28–3.16)	3.66 (2.45–5.46)					
	8-14	1.28 (0.83–1.97)	2.22 (1.50–3.29)					
	15-28	1.39 (1.03–1.89)	1.44 (1.01–2.06)					
	29-91	0.99 (0.82–1.19)	1.16 (0.94–1.43)					
	Baseline	1.00	1.00					
		<60 years	60-70 years	p	70-80 years	p	>80 years	p
By age group*	1-3	1.46 (0.47–4.55)	3.93 (2.15–7.18)	0.13	4.14 (2.47–6.95)	0.10	5.94 (3.90–9.04)	0.023
	4-7	1.46 (0.54–3.91)	1.89 (0.89–4.00)		3.55 (2.18–5.78)		3.18 (1.93–5.25)	
	8-14	1.88 (0.97–3.65)	1.09 (0.51–2.30)		2.31 (1.45–3.66)		1.40 (0.79–2.48)	
	15-28	1.50 (0.88–2.56)	0.96 (0.54–1.71)		1.81 (1.23–2.65)		1.35 (0.88–2.07)	
	29-91	0.84 (0.59–1.21)	1.03 (0.77–1.38)		0.96 (0.73–1.26)		1.31 (1.04–1.66)	
	Baseline	1.00	1.00		1.00		1.00	
		STEMI	NSTEMI	p				
By infarction type	1-3	4.66 (3.04–7.15)	3.89 (2.71–5.60)	0.53				
	4-7	1.76 (0.97–3.21)	3.25 (2.30–4.60)					
	8-14	1.77 (1.12–2.80)	1.60 (1.10–2.33)					
	15-28	1.13 (0.74–1.71)	1.58 (1.20–2.09)					
	29-91	0.98 (0.78–1.23)	1.10 (0.93–1.31)					
	Baseline	1.00	1.00					
		No	Yes					
By history of previous vascular disease	1-3	4.32 (3.10-6.02)	3.89 (2.35-6.42)	0.73				
	4-7	3.00 (2.12-4.25)	2.03 (1.11-3.69)					
	8-14	1.68 (1.18-2.39)	1.62 (0.97-2.72)					
	15-28	1.37 (1.03-1.82)	1.49 (1.00-2.21)					
	29-91	0.99 (0.83-1.17)	1.19 (0.95-1.51)					
	Baseline	1.00	1.00					

Table 5.3 Adjusted IRs for AMI occurring after ARI overall and stratified by gender and age group

[±]Number of AMI events occurring in each risk period

∞p values presented from Cochran Q test for heterogeneity in incidence ratios for AMI in days 1-3 after acute respiratory infection between different strata.
*For each age group, incidence ratios for AMI in days 1-3 after acute respiratory infection are compared with the result for <60 years

5.5.4 Sensitivity analyses

Results of sensitivity analyses are shown in table 5.4. Effect sizes did not change markedly between different analyses including restricting to AMI events where the patient did not die in hospital – adjusted IR 3.56 (95% CI 2.59 - 4.90) for days 1–3.

Analysis of AMI risk	Cases included in analysis, n	Age– and season–adjusted incidence ratio (95% CI)
Initial analysis		
All first AMIs after acute respiratory infection	3,927	4.19 (3.18–5.53)
Sensitivity analyses		
Adjusting for age in 1 year age-bands	3,927	4.14 (3.14–5.46)
Excluding events where patient died in hospital	3,548	3.56 (2.59–4.90)
Excluding events with symptom date inferred from discharge date and average length of stay	3,870	4.27 (3.24–5.63)
Including events with missing discharge diagnosis	4,089	3.99 (3.03–5.27)

Table 5.4 Adjusted IRs for AMI occurring in days 1-3 after ARI overall and then under various sensitivity analyses

5.5.5 Effect of general acute respiratory infection on secondary outcomes (HES- and GPRD-recorded AMI)

5.5.5.i AMI recorded in HES

15,185 patients aged ≥40 years had a record of first AMI in HES during the study period. Of these patients, 5,420 (35.7%) had also consulted for an acute respiratory infection during this time period (mean number of consultations 2.1 per person among those with at least one consultation record) so were eligible for inclusion in self-controlled case series analysis. 56.9% of patients included in analysis were male with a median age of 75.0 years (IQR 63.8 - 82.9 years).

The age- and season- adjusted incidence ratio for HES-recorded AMI occurring 1-3 days after consultation for acute respiratory infection was 6.07 (95% CI 4.99 - 7.38). As before incidence ratios tapered over time: IR 2.92 (95% CI 2.29 - 3.73) for days 4-7; IR 2.41 (95% CI 1.96 - 2.96) for days 8-14; IR 1.55 (95% CI 1.29 - 1.87) for days 15-28; IR 1.17 (95% CI 1.05 - 1.32) for days 29-91.

5.5.5.ii AMI recorded in linked GPRD records

14,867 patients aged ≥40 years had a record of first AMI in GPRD during the study period. Of these, 5,502 had a record of acute respiratory infection (mean 2.1 per person) so were included in self-controlled case series analysis. 58.0% were male with a median age of 73.6 years (IQR 62.8 - 81.8 years).

The age- and season- adjusted incidence ratio for GPRD-recorded AMI occurring 1-3 days after consultation for acute respiratory infection was 3.16 (95% CI 2.41 - 4.15). Incidence ratios fell over time – IR 2.04 (95% CI 1.52 - 2.74) for days 4-7; IR 2.15 (95% CI 1.73 - 2.68) for days 8-14; IR 1.68 (95% CI 1.40 - 2.01) for days 15-28; IR 1.25 (95% CI 1.12 - 1.39) for days 29-91.

Results for the same analysis using MINAP-recorded AMI (the primary outcome) compared to AMI recorded in HES and GPRD are shown in table 5.5.

Risk period after infection (days)	**Age– and season– adjusted IR (95% CI)**		
	MINAP (n=3,927)	HES (n=5,420)	GPRD (n=5,502)
1-3	4.19 (3.18–5.53)	6.07 (4.99-7.38)	3.16 (2.41-4.15)
4-7	2.69 (1.99–3.63)	2.92 (2.29-3.73)	2.04 (1.52-2.74)
8-14	1.66 (1.24–2.23)	2.41 (1.96-2.96)	2.15 (1.73-2.68)
15-28	1.41 (1.12–1.77)	1.55 (1.29-1.87)	1.68 (1.40-2.01)
29-91	1.05 (0.92–1.21)	1.17 (1.05-1.32)	1.25 (1.12-1.39)
Baseline	1.00	1.00	1.00

Table 5.5 Comparison between risks of AMI recorded in MINAP, HES and GPRD after ARI

5.5.6 Comparison of effects of ARI episodes judged more and less likely to be due to influenza on primary outcome (MINAP-recorded AMI)

There was a higher incidence ratio for AMI after acute respiratory infections occurring during peak weeks of influenza circulation compared to non-peak weeks (p varied from 0.006 to 0.091 depending on the definition of peak weeks

used). Though the point estimate for episodes coded as influenza-like illness was nearly double that of episodes coded as general acute respiratory infection – IR 7.31 (95% CI 2.72 - 19.64) versus IR 4.03 (95% CI 3.02 - 5.38) for 1–3 days – this difference was not statistically significant (p=0.26). Unvaccinated episodes (thought to be most likely to represent influenza infections) were associated with similar incidence ratios to episodes for which there was likely to be residual vaccine protection (p=0.94) and to vaccinated episodes (p=0.91). Overall comparing acute respiratory infections with at least one indicator of influenza to those with no indicators suggested a significantly greater effect on AMI – IR 5.39 (95% CI 3.89 - 7.45) compared to IR 2.38 (95% CI 1.37 - 4.11) for 1–3 days (p=0.012) (table 5.6). This finding is also shown graphically in figure 5.3.

Risk period after infection (days)	**"Influenza" indicator (N=no. of acute respiratory infection records)** **Age– and season–adjusted incidence ratio (95% CI)**		
	Peak weeks[1] (N=1,278)	Non peak weeks (N=6,847)	p
1-3	7.79 (4.71–12.90)	3.32 (2.37–4.65)	0.006
4-7	1.86 (0.77–4.51)	2.80 (2.04–3.86)	
8-14	0.87 (0.33–2.34)	1.79 (1.32–2.42)	
15-28	1.69 (1.01–2.82)	1.35 (1.05–1.74)	
29-91	0.89 (0.62–1.27)	1.08 (0.93–1.25)	
Baseline	1.00	1.00	
	Peak weeks[2] (N=1,733)	Non peak weeks (N=6,392)	p
1-3	5.95 (3.60–9.81)	3.54 (2.53–4.95)	0.091
4-7	3.68 (2.12–6.40)	2.37 (1.66–3.39)	
8-14	0.99 (0.44–2.23)	1.82 (1.33–2.48)	
15-28	1.54 (0.96–2.47)	1.37 (1.06–1.78)	
29-91	1.02 (0.76–1.36)	1.09 (0.94–1.26)	
Baseline	1.00	1.00	
	Peak weeks[3] (N=2,226)	Non peak weeks (N=5,899)	p
1-3	6.34 (4.13–9.72)	3.16 (2.19–4.57)	0.016
4-7	3.07 (1.80–5.22)	2.48 (1.73–3.56)	
8-14	0.89 (0.42–1.88)	1.92 (1.40–2.63)	
15-28	1.32 (0.85–2.07)	1.43 (1.10–1.86)	
29-91	1.06 (0.82–1.36)	1.05 (0.90–1.23)	
Baseline	1.00	1.00	

	ILI code (N=410)	General code (N=7,796)	p				
1-3	7.31 (2.72–19.64)	4.03 (3.02–5.38)	0.26				
4-7	1.37 (0.19–9.74)	2.61 (1.91–3.57)					
8-14	1.56 (0.39–6.28)	1.66 (1.24–2.24)					
15-28	0.79 (0.20–3.16)	1.43 (1.13–1.81)					
29-91	1.17 (0.67–2.05)	1.06 (0.92–1.23)					
Baseline	1.00	1.00					
	Unvaccinated (N=1,590)	Residual protect-ion (N=1,466)	p	Vaccinated (N=5,069)	p		
1-3	4.15 (2.20–7.83)	4.30 (2.29–8.07)	0.94	3.99 (2.79–5.70)	0.91		
4-7	1.90 (0.85–4.29)	2.62 (1.29–5.26)		2.92 (2.03–4.20)			
8-14	1.87 (0.99–3.51)	2.26 (1.27–4.03)		1.41 (0.95–2.09)			
15-28	1.91 (1.21–3.03)	0.58 (0.26–1.31)		1.50 (1.13–1.98)			
29-91	1.08 (0.79–1.46)	1.15 (0.86–1.55)		1.05 (0.88–1.25)			
Baseline	1.00	1.00		1.00			
	0 indicators∞ (N=3,433)	1 indicator (N= 3,771)	p	2 or 3* (N=921)	p	At least 1 (N=4,692)	p
1-3	2.38 (1.37–4.11)	5.53 (3.88–7.88)	0.011	4.31 (1.92–9.68)	0.23	5.39 (3.89–7.45)	0.012
4-7	2.49 (1.56–3.97)	2.76 (1.79–4.26)		2.73 (1.13–6.60)		2.80 (1.89–4.13)	
8-14	1.67 (1.08–2.58)	1.61 (1.04–2.48)		1.58 (0.65–3.82)		1.63 (1.10–2.40)	
15-28	1.61 (1.17–2.23)	1.12 (0.77–1.63)		1.64 (0.87–3.07)		1.21 (0.87–1.68)	
29-91	1.07 (0.88–1.31)	1.03 (0.85–1.26)		1.08 (0.73–1.59)		1.05 (0.88–1.26)	
Baseline	1.00	1.00		1.00		1.00	

Table 5.6 Adjusted IRs for AMI after ARI episodes with and without indicators of influenza

[1] Peak weeks of influenza circulation defined using two weeks either side of peak week

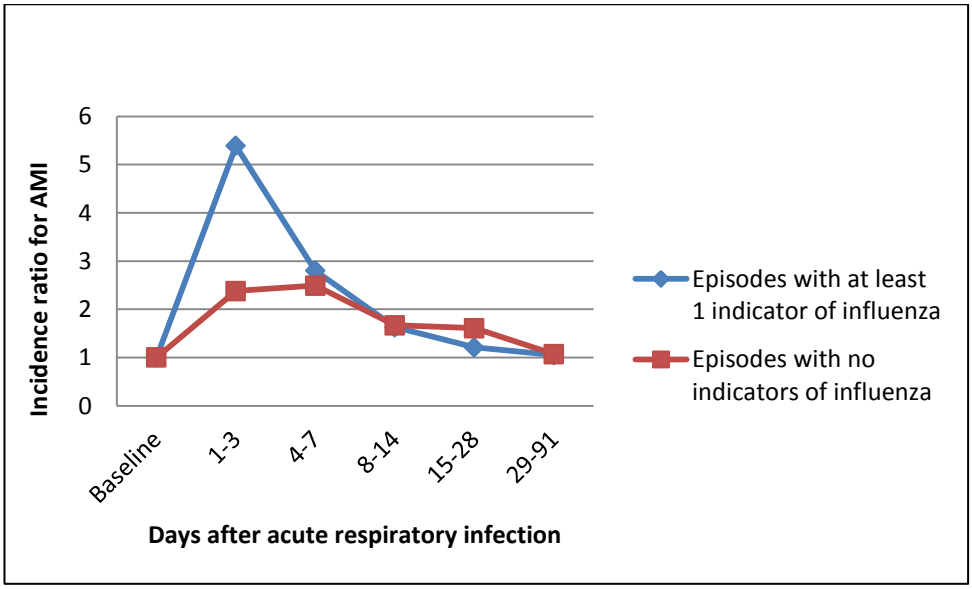
[2] Peak weeks = 3 weeks either side of peak week

[3] Peak weeks = 4 weeks either side of peak week

∞ Indicators of influenza were occurrence of acute respiratory infection during a peak week (using definition 3), presence of an ILI code and unvaccinated status

* 2 or 3 indicators pooled due to small numbers

Figure 5.3 Graph of adjusted IR for MINAP-recorded AMI after ARIs with at least 1 indicator of influenza (blue) and with no influenza indicators (red)



5.6 Discussion

5.6.1 Summary of findings

This study, one of the first to use the new GPRD-MINAP data linkage, showed a short-term increased risk of AMI in days following acute respiratory infection, confirming previous findings using GPRD data alone (chapter 4). The effect was greatest for people aged over 80 years. Episodes of acute respiratory infection occurring when seasonal influenza virus was circulating had significantly higher incidence ratios than illnesses occurring in other time periods.

5.6.2 Study strengths

A major strength of this study was the use of a novel data linkage to improve the accuracy and timing of data on AMI. Previous validation studies of AMI in the GPRD have found that although AMI diagnoses have a generally high positive predictive value[149], less is known about the accuracy of recording of timing[168]. In one validation study, 15% of confirmed cases had a different date of AMI recorded in GPRD to that provided by the GP[169]. For self-controlled case series

analysis using short risk periods, even a small proportion of AMI records with inaccurate dates or false positive records could introduce considerable bias. Therefore I used MINAP data, which has the advantage of multiple data entry fields for information on timing of AMI to allow internal validation of the AMI date as well as improved diagnostic precision. Findings were confirmed in HES, which is another independently-recorded source of information on timing of AMI. Using improved data on timing of AMI also increased the precision of stratified analyses using influenza surveillance data. While acute respiratory infections can be accurately categorised as occurring either in peak or non-peak periods of influenza circulation, delayed recording of AMI (such as may occur when using GPRD data alone) would diminish any effect seen.

Using self-controlled case series eliminated any fixed between-person confounding that may have affected similar analyses using case control or cohort designs[170]. Using within-person comparisons also reduced the risk of residual bias due to difficulties choosing appropriate controls. While AMI is an event that can potentially affect the length of the observation period (which goes against the assumptions on which the case series method is based[171]), when I excluded AMI events where the patient died in hospital this did not significantly alter results.

5.6.3 Study limitations – diagnosing influenza in primary care

As previously noted in chapter 4, influenza infections in primary care are rarely microbiologically confirmed. Therefore in this study several methods were used to divide acute respiratory infections into those more and less likely to be due to influenza. As in chapter 4, I used medical codes entered by GPs to classify respiratory infections. While the effect size was substantially higher for ILIs compared to other acute respiratory infections, the difference between groups was not significant; this analysis was underpowered to detect an effect. In this study two additional methods were used to identify illnesses likely to be caused by influenza. The more powerful was to use linked influenza surveillance data, which showed a greater effect in peak weeks of influenza circulation than non-peak weeks, strongly suggesting that influenza infections are more likely to trigger AMI than other respiratory infections. Varying the number of weeks

included in the 'peak' and 'non-peak' periods resulted in similar magnitudes of effect. When this analysis was done in GPRD alone (chapter 4) no significant difference was seen between illnesses occurring in peak and non-peak periods, but this was likely to be due to inaccurate data on timing of AMI.

In this study I also used influenza vaccination data to compare 'vaccinated' against 'unvaccinated' illnesses but did not see a difference in effect on AMI. This does not mean that influenza vaccination is ineffective at preventing influenza-associated AMI; rather, acute respiratory infections occurring after influenza vaccination are likely to have a different aetiology. It is also possible that immunisation status was misclassified for some illnesses: I did not have data on vaccinations occurring in other settings eg the workplace; influenza vaccine effectiveness is around 70% in healthy adults and lower in frail elderly populations[172]. These results suggest that acute respiratory infections other than influenza can also be associated with increased AMI risk. Combining these methods, though, I found significantly higher incidence ratios for illnesses with at least one influenza indicator compared to those with no indicators of influenza. One final caveat when using primary care data to obtain information on acute respiratory infections is that one is reliant on the GP's diagnosis. It is possible that some symptoms such as chest pain and shortness of breath diagnosed as acute respiratory infections actually heralded gradually evolving myocardial loss. Incidence ratios would be artificially inflated by any such misclassification.

5.6.4 Interpretation of results in context of previous findings

The main result from this analysis using linked data – an adjusted incidence ratio of 4.19 (95% CI 3.18 - 5.53) for AMI occurring 1-3 days after acute respiratory infection – was higher than that seen in the previous chapter using GPRD data alone – IR 3.65 (95% CI 3.10 - 4.30). This is perhaps unsurprising as using linked data to improve information on AMI timing is likely to have reduced the risk of retrospective recording of events that may have occurred in GPRD and would dilute effect sizes seen. Additional confirmation of this result was provided by using the secondary outcome HES-recorded AMI, for which the adjusted incidence ratio was even higher – IR 6.07 (95% CI 4.99 - 7.38). This study provided some

evidence that the triggering effect of acute respiratory infection on AMI was stronger for influenza than for other infections. This is supported by influenza vaccine studies, which provide indirect evidence for a specific triggering effect of influenza. From our previous review, pooled analysis of data from three relatively small randomised controlled trials in populations with existing cardiovascular disease[102,105,126] suggested that influenza vaccine is associated with protection against cardiovascular death – relative risk 0.46 (95% CI 0.21 - 1.02) – as well as a 33% reduction in risk of AMI. Given the potential difficulty of capturing confirmed influenza infections accurately in AMI patients, these studies might provide the best available evidence of a specific influenza effect.

5.6.5 Implications for policy and practice

This study provides further evidence that seasonal influenza has a stronger triggering effect on AMI than some other acute respiratory infections. Therefore findings support increased efforts to maximise uptake of influenza vaccination in elderly people and cardiovascular risk groups to protect against cardiovascular as well as more obvious respiratory complications of influenza. This analysis was restricted to first AMI events. People who had these would not necessarily fall under the vaccination guidelines as people with established cardiac disease. Although we found similar effect sizes in people with and without evidence of previous vascular disease using a broad definition, further work on delineating high-risk groups is needed. In particular, further trial evidence is needed on the use of influenza vaccine for primary prevention of cardiovascular events in previously healthy and at-risk populations. This is a potential argument for lowering the age limit for routine influenza vaccination (currently 65 years in the UK) to cover younger age groups at risk of cardiovascular disease. Nonetheless as the triggering effect was greatest in older adults, it is recognised that such a strategy may not be cost-effective.

5.6.6 Future directions

In studies using primary care data, lack of microbiological confirmation of diagnosis remains an issue when investigating infections such as influenza for

which laboratory testing is not routinely done. In future, studies linking primary care data on consultations with laboratory data eg from the HPA's 'Lab base' database of positive laboratory specimens would help to elucidate the relative importance of influenza as an AMI trigger compared to other acute respiratory infections. Chapter 6 describes a primary case control study conducted in patients hospitalised with AMI during the 2009 influenza A H1N1 pandemic in which influenza testing is built into the protocol.

SUMMARY

- Self-controlled case series analysis from the previous chapter was repeated using linked data from GPRD, MINAP and HES to enhance the accuracy and timing of data on AMI.
- In the time period 01/01/2003-31/07/2009 there were 11,208 people with a first AMI recorded in MINAP of whom 3,927 had also consulted for acute respiratory infection
- An even higher AMI risk than before was apparent in the first three days after GP consultation for acute respiratory infection – adjusted IR 4.19 (95% CI 3.18 - 5.53).
- The effect was greatest for people aged over 80 years.
- Episodes of acute respiratory infection occurring when influenza virus was circulating had significantly higher incidence ratios than illnesses occurring in other time periods, providing some evidence that a triggering effect on AMI might be stronger for influenza than other acute respiratory infections.

6. Influenza-like illness in acute myocardial infarction patients during the winter wave of the influenza A H1N1 pandemic: a hospital-based case control study

6.1 Description of chapter contents

This chapter describes a case control study investigating the experience of recent respiratory and influenza-like illness in adults hospitalised with AMI during the second wave of the 2009 influenza A H1N1 pandemic in London compared to adults hospitalised for acute non-vascular surgical conditions. The main exposure is clinically-defined ILI. Additional exposures are nasopharyngeal and throat swabs testing positive for influenza by real-time polymerase chain reaction and presence of IgA antibodies to influenza A in serum samples. Logistic regression models are generated to investigate likelihood of recent infection in cases compared to controls. Findings are placed into context with results from other pandemic and seasonal influenza studies.

6.2 Introduction and study rationale

6.2.1 2009 influenza A H1N1 pandemic

As evidenced by work described in chapters 2-5, seasonal influenza can trigger cardiovascular complications and deaths, especially in elderly people. In a pandemic situation, however, when there is global spread of a novel influenza strain, clinical and demographic profiles of those affected may change dramatically. The most recent influenza pandemic was caused by an influenza A H1N1 strain (H1N1pdm09) that emerged in Mexico and the United States in April 2009[173,174]. The UK experienced several waves of infection with this novel strain - a first wave occurred in spring and summer 2009 followed by a second wave in the winter of 2009/10 and a post-pandemic wave in winter 2010/11[175]. Initial evidence from the first wave in the UK suggested that typical illnesses were mild and affected mainly children and young people[176]. The average age of cases increased over subsequent waves of the pandemic[177] but it is unclear how this

affected clinical illness profiles. Vaccination coverage did not reach high levels until the post-pandemic season.

6.2.2 Effect of influenza pandemic on AMI

There have been reports of myocarditis, myocardial injury and left ventricular systolic dysfunction in patients with severe H1N1pdm09[178,179]. It has been suggested that H1N1pdm09 was associated with higher rates of extra-pulmonary complications than seasonal influenza[180] but this is difficult to compare as surveillance of severe influenza-related disease was greatly enhanced during the pandemic. A recent mathematical modelling study estimated that globally there were 83,300 cardiovascular deaths associated with the first twelve months of H1N1pdm09 circulation in adults aged >17 years[121], but the contribution of myocardial infarction deaths to this figure is unknown.

6.2.3 Study rationale

In this study I aimed to investigate whether a relationship was evident between the pandemic influenza strain H1N1pdm09 and AMI, to extend work in previous chapters covering time periods of seasonal influenza circulation. While using primary care records (as for studies described in chapters 4 and 5) has many advantages, a potential limitation is lack of information on factors not routinely measured in primary care such as laboratory confirmation of influenza. To address the question of whether influenza virus was more strongly associated with AMI than other respiratory viruses, accurate diagnosis is essential. Therefore I decided to conduct a primary study to measure patients' influenza status directly. A case control study was the most feasible design to allow data to be collected relatively quickly and with limited resources on (theoretically) sufficient numbers of patients with AMI. The following sections describe methods to measure influenza for clinical and research purposes.

6.2.4 Clinical definitions of influenza

Identifying episodes of true influenza infection is challenging: it is recognised that the community burden of influenza is substantially underestimated in health service data as most patients with influenza do not seek medical care. Patients who do present with symptoms of influenza rarely have microbiological testing and confirmation of diagnosis[181]. While various clinical diagnostic criteria exist for influenza-like illness[182,183], these criteria may correlate poorly with laboratory-confirmed influenza[184]. In a study of 100 adult patients with PCR-confirmed influenza A H1N1 (pandemic strain), the WHO definition of influenza-like illness (acute fever >38°C and cough or sore throat in the absence of another diagnosis) had only 50% sensitivity and 87% specificity for differentiating influenza from general acute respiratory infections[185]. The accuracy of clinical case definitions for diagnosing influenza varies depending on patient factors such as age, presence of underlying disease and vaccination status as well as external factors such as levels of influenza circulation[181]. In chapters 4 and 5 these factors were explored using stratified analyses.

6.2.5 Laboratory definitions of influenza

Laboratory tests for influenza typically detect either the influenza virus or the patient's immune response to it[186]. Tests range from 'point of care' tests designed to detect influenza virus at the bedside (eg rapid antigen tests based on enzyme immunoassays) to nucleic acid testing using reverse-transcriptase polymerase chain reaction, to virus culture (the traditional 'Gold standard' although it has lower sensitivity than PCR-based methods) and finally detection of influenza antibodies in sera[187]. The choice of test is influenced both by laboratory factors such as size, capacity, infrastructure and training of laboratory personnel[186] as well as clinical or research need, eg the rapidity of diagnosis required. For this case control study requirements were different to those for clinical diagnosis: it was suspected that most participants would not have symptoms of influenza infection at the point of testing. Rather it was hypothesised that more patients admitted with AMI than control patients may have experienced influenza within the last month. Any test designed to detect influenza virus nucleic acid or

proteins used alone would have low sensitivity for identifying recent past infections. I therefore focussed additionally on detection of influenza antibodies in sera.

6.2.6 Serological diagnosis of influenza

Traditional serological diagnosis of influenza is based on a fourfold or greater rise in specific antibody titre in paired serum samples, the first taken as soon as possible after symptom onset and the second around 10-14 days later[37]. Tests include virus neutralisation, haemagglutinin inhibition, complement fixation, enzyme immunoassays and indirect immunofluorescence microscopy[186]. Approximately 80% of subjects demonstrate a protective serum antibody response (haemagglutinin inhibition titre>40) after natural influenza infection[188]. The main antibody isotypes comprising the humoral immune response to influenza are IgM, IgA and IgG[189]. During primary infections, IgM and IgA levels peak after 2 weeks and then begin to decline, while serum antibodies of the IgG subclass peak at 4-6 weeks[188]. IgM antibodies initiate complement-mediated neutralisation of the virus and are a hallmark of the primary immune response[190]. IgA levels rise after both primary and secondary infections[191]. IgG antibodies are present in both primary and secondary immune responses and afford long-lived protection in the respiratory tract[192].

Several investigators have sought assays that determine IgM, IgG and IgA separately from a single serum sample rather than from paired samples. In a cohort of patients with influenza A confirmed by either virus culture or direct immunofluorescence assay within 36 hours of symptom onset, IgA antibodies were detected in 68% on a single serum assay, compared to 45% for IgM antibodies and 62% for IgG[191]. Virus-specific IgA and the bulk of IgG was synthesised within the first week. Authors conclude that finding specific serum IgA is highly indicative of a recent influenza infection and should be the method of choice after seven days (when virus itself can no longer be isolated).

The combination of clinical and laboratory methods used to diagnose influenza in this study is explained in section 6.4.5.

6.3 Aims and objectives

Aim: To conduct a case control study investigating the experience of recent influenza infection in patients hospitalised with AMI during the second wave of the 2009 influenza A H1N1 pandemic in London compared to adults hospitalised for acute non vascular surgical conditions.

Objectives

1. To investigate whether AMI patients were more likely to have had recent ILI (primary exposure) than patients with acute surgical conditions during the second wave of the influenza A H1N1 pandemic.
2. To investigate whether AMI patients were more likely to have had recent respiratory illness or concurrent PCR positive influenza or evidence of influenza A IgA antibodies in sera (secondary exposures) than patients with acute surgical conditions.
3. To examine whether influenza vaccination was associated with protection against AMI.

Supplementary objective

1. To examine the feasibility and sample size needed to conduct a full scale case control study in the event of under-recruitment.

6.4 Methods

6.4.1 Setting and design

This was an observational case control study carried out in hospital in-patients at the Royal Free London NHS Foundation Trust between 21st September 2009 and 28th February 2010. These dates roughly corresponded to the second (winter) wave of H1N1pdm09 circulation.

6.4.2 Participants

Cases were patients aged ≥40 years admitted with an acute myocardial infarction (defined as a rise in troponin T with ischaemic symptoms and/or typical ECG changes, or by angiographic evidence of acute coronary artery thrombosis during primary percutaneous coronary intervention). Controls were patients aged ≥40 years admitted with an acute surgical condition such as appendicitis, bowel or urinary obstruction and no history of AMI within the past month, frequency-matched for gender, age-group in 5 year age-bands and week of admission. All were English-speaking and able to provide written informed consent.

6.4.3 Procedures for recruitment and obtaining consent

Participants were recruited from the acute cardiology ward and coronary care unit (cases) and from ward 9 North A, an acute surgical ward (controls), with the aid of their respective clinical teams. Potentially eligible cases were identified by the cardiac clinical audit nurse in three ways: 1) through obtaining a list of names of patients on the cardiac catheter list, 2) through reviewing a list of names of patients on the cardiac ward from the nurses' handover and 3) through examining a list of all patients in the hospital with a positive troponin result and checking whether their medical notes recorded a diagnosis of acute coronary syndrome. I was given a list of names of potential cases and discussed whether they met eligibility criteria with the cardiology ward registrar. For recruitment of controls, I met the charge nurse on the acute surgical admissions ward every morning to be given names of potential participants meeting inclusion criteria.

Patients who were eligible to take part were given a participant information sheet by ward nurses. I returned several hours later to discuss the study's aims and objectives, methods, potential risks and benefits with potential participants who had agreed to be approached and answered any questions. Those who agreed to participate signed a study consent form. Examples of the participant information sheet and consent form are given in appendix 10.1.4.

6.4.4 Exposures

The main exposure was recent ILI, defined as a history of feeling feverish with either cough or sore throat within the last month. I also used the exposure recent acute respiratory infection to capture a history of respiratory illness within the last month with any of the following symptoms – fever, chills, dry cough, productive cough, myalgia, rhinorrhoea, blocked nose, sore throat, wheeze, earache and fatigue – that did not meet ILI criteria. Additional exposures were nasopharyngeal and throat swabs testing positive for influenza by real-time polymerase chain reaction, presence of IgA antibodies to influenza A in serum samples (concentration>12U/ml defined positivity) and self-reported influenza vaccination status.

6.4.5 Data sources and measurement

6.4.5.i Questionnaire and medical records

Data sources and information collected are summarised in table 6.1. Briefly, I used a questionnaire to investigate recent respiratory and influenza-like illness and to collect data on demographics, medical history and influenza vaccination status. I reviewed medical records for details of the current admission and, where possible, verified information on confounding factors.

6.4.5.ii Serum samples

A single serum sample was taken for quantification of IgA antibodies to influenza A as a marker of recent exposure. If a patient was being discharged the same day as recruitment a sample was taken immediately, or otherwise requested from the ward phlebotomist for the following day. Serum samples were transported, spun down, frozen at -80^{0}C and batch tested in the Virology laboratory of the Royal Free Hospital using a commercially available enzyme-linked immunosorbent assay (ELISA) for influenza A IgA (*Biosupply UK, cat no. RE56501*). The ELISA was based upon the 'sandwich principle' whereby wells were coated with antigen, allowing specific antibodies from serum samples to bind to these antigen-coated wells. These were detected by a secondary enzyme-conjugated antibody specific for human IgA. The intensity of colour produced during the substrate reaction

was proportional to the amount of IgA-specific antibodies detected. Results were read off a standard curve. Any samples with equivocal results were repeated.

A test based on a single sample was chosen partly for logistic reasons and partly because, in participants with recent (rather than current) influenza, it was also likely that an antibody response would already be maximal on the first test so a repeat serum sample would not necessarily demonstrate an increasing titre.

6.4.5.iii Respiratory samples

Combined nasopharyngeal and throat swabs were collected on the day of recruitment, placed in viral transport medium and transported to the Virology laboratory for storage at -80^{0}C. Samples were tested in batches of 25 for the presence of influenza virus RNA using a validated in house real-time polymerase chain reaction with a lower limit of detection of 1 RNA copy per reaction[193].

Type of information	Characteristic	Type of variable	Data source
Demographic data	Age Gender Ethnicity Marital status Employment status Years of education	Potential confounders	Patient questionnaire
Cardiac risk factor data	Smoking status Diabetes Hypertension Hypercholesterolaemia Family history of AMI Past history of AMI BMI	Potential confounders	Patient questionnaire/ Medical records
Respiratory illness data	Any symptoms of respiratory illness before admission? Y/N When? 1-3/ 4-7/ 8-14/ 15-28 days Fever? Chills? Cough – dry? Cough – productive? Myalgia? Runny nose? Blocked nose? Sore throat? Wheeze? Earache? Fatigue? Influenza vaccination status?	Exposure	Patient questionnaire
Serological evidence of recent influenza	Influenza A IgA	Exposure	Serum sample
PCR evidence of recent influenza	Influenza virus RNA detected by real-time PCR	Exposure	Combined nasopharyngeal and throat swab
Evidence of current condition	AMI or acute surgical condition	Outcome	Medical records (clinical history and results of imaging and biochemical investigations)

Table 6.1 Sources of data collected from cases and controls

6.4.6 Approaches to minimise bias and confounding

6.4.6.i Selection bias

While appropriate selection of controls was critical to reducing selection bias, a pragmatic decision was also necessary for logistical and cost purposes. Hospital-based controls were chosen partly for feasibility of recruitment but with the assumption that they would have been subject to the same selective forces as the case group. Both potential cases and controls transferred from other hospitals were excluded. The source population therefore comprised patients who would seek treatment at the Royal Free Hospital if acutely unwell rather than corresponding to the population of the local geographic area. Acute surgical admissions were chosen over elective admissions as elective admissions would be unrepresentative of exposure distribution in the source population (ie anyone with influenza would have their operation postponed). In addition, acute vascular surgical admissions were not included as controls because the underlying pathophysiological process leading to acute admission was likely to be similar to that resulting in AMI.

6.4.6ii Information bias

Information bias was reduced through using a standard checklist of questions for both cases and controls to obtain information on demographics, cardiac risk factors, details of any recent respiratory illness and influenza vaccination status. I developed clear wording to introduce the study to patients and carefully drafted participant information sheets to reduce the risk of recall bias. As I conducted interviews myself it was not possible to be blind to the case or control status of participants. Nevertheless I verified information collected at interview using data from medical records extracted with a standard data extraction sheet to reduce information bias through misclassification of exposure or outcome data. Samples were analysed using batch testing and standardised assay procedures so that laboratory scientists were generally unaware of the case or control status of the patient. A Microsoft Access database was designed to store results. This had appropriate ranges entered into all fields where answers could be categorized to reduce the risk of data entry errors.

6.4.6.iii Confounding

Frequency matching on gender, age-group and week of admission helped to reduce baseline imbalances between groups and to control for factors that may confound an association between acute respiratory infection and AMI.
Information on a range of potential confounders such as smoking status was also collected so that these could be controlled for in multivariable statistical models.

6.4.7 Study size considerations

6.4.7.i Sample size calculations

This study was planned before the advent of the 2009 influenza A H1N1 pandemic so sample size calculations were based on seasonal influenza data. Sample size calculations were performed using the following formula:

$$n \text{ is } > [u\sqrt{(\pi 0(1-\pi 0) + \pi 1(1-\pi 1))} + v\sqrt{(2\pi(1-\pi))}]^2 / (\pi 1-\pi 0)^2$$

where n gives the number of cases needed and an identical number of controls is also required. Table 6.2 gives an explanation of variables used in this formula.

Variable	Explanation of variable	Calculation of variable (if applicable)	Value
π0	Proportion of controls exposed	From the Flu Watch study influenza seroconversion rate = 22.5% over 4.5 months = 0.225/4.5 or 0.05 per month	0.05
OR	Odds ratio	Based on incidence ratios calculated in chapters 4 and 5 - IR 3.65 (95% CI 3.10-4.30) & IR 4.19 (95% CI 3.18–5.53)	3
π1	Proportion of cases exposed	Calculated from: π1= (π0*OR)/1+ π0(OR-1)	0.095238095
π	Proportion of participants exposed	(π1 + π0)/ 2	0.072619047
u	Power	One-sided percentage point of the normal distribution corresponding to 100% minus the power (ie 90% power)	1.28
v	Significance level	Percentage point of the normal distribution corresponding to the two-sided significance level (ie 5% significance)	1.96

Table 6.2 Explanation of variables included in sample size calculation

Table 6.3 shows the sample size needed for varying levels of power and significance, with a case: control ratio of 1:1 to detect an odds ratio of 3 in an unmatched case control study. For 90% power and 5% significance I estimated that 236 cases and 236 controls would be required.

Significance	Power		
	80%	90%	95%
0.1	N=101 (x2)	N= 147(x2)	N= 235(x2)
0.05	N=176 (x2)	N= 236 (x2)	N= 344(x2)
0.01	N=262 (x2)	N= 333(x2)	N= 461(x2)

Table 6.3 Sample sizes needed to detect an odds ratio of 3 with varying power and significance

6.4.7.ii Expected numbers of eligible cases

The Royal Free hospital typically admits around 200 STEMI patients per year and at least 200 patients with NSTEMI. More patients are admitted with AMI in winter than summer. Of the STEMI patients around 30-33% are transferred from other hospitals for angiography (so would not be eligible for inclusion), 20% arrive through A&E and 50% direct from the London ambulance service. All NSTEMI patients would theoretically be eligible as they tend not to be transferred from other hospitals. In total approximately 240 AMI patients are admitted in six months over the winter, of whom around 200 would be eligible for inclusion (assuming that all NSTEMI and 70% of STEMI patients would be eligible).

6.4.7.iii Expected numbers of eligible controls

At the Royal Free Hospital, across all surgical teams and assuming a fairly high attrition rate, it is estimated that 10-20 acute surgical patients aged ≥40 years could be recruited per week. This is based on the average number of emergency/ semi emergency admissions on an acute surgical take being 8-15 patients of all ages, recognising that many will be discharged rapidly or will not be available to participate due to undergoing investigations or surgical treatments. In total there would be around 390 eligible acute surgical patients in six months over the winter, assuming a mean of 15 eligible admissions per week

6.4.7.iv Expected length of recruitment period

To recruit 236 cases and 236 controls from a single centre, I anticipated that recruitment would need to continue over two influenza seasons. The timing of recruitment was planned to coincide with the timing of the influenza season. This can be unpredictable, especially in the event of an influenza pandemic, so start and end dates were flexible.

6.4.7.v Expected effect of missing data

Efforts were made to minimise missing data on cardiac risk factors and demographic factors by correlating answers to questionnaires with data from medical records. It was expected that not all patients who provided questionnaire data would have a usable serum sample (due to factors such as missing serum samples or possible technical difficulties with the assay). Use of

both clinical and virological endpoints did, however, allow inclusion of these participants' data in analyses even if some laboratory data were lacking.

6.4.8 Data management and coding

6.4.8.i Data entry

Data from questionnaires and medical records were collected on paper forms and entered into a purpose-built Microsoft Access database under a participant's study identification number. Written records were kept securely in a file in a locked filing cabinet in a locked university office. Laboratory data were emailed from the virology laboratory and results entered into the database by study identification number. The Access database was stored securely on the University server, which is backed up daily. 'Look up tables' were created on the database to correspond to answers from questionnaires with the aim of reducing data entry errors. Reducing the amount of free text entered also helped with categorisation of variables for use in generating frequency tables and in analyses.

6.4.8.ii Classification of outcome and exposure

The outcome, AMI, was categorised as STEMI, NSTEMI or myocardial infarction not otherwise specified. Categories were combined to maximise power for the main analysis. PCR and clinical exposure variables were binary, with codes corresponding to the presence or absence of influenza virus on swabs, recent respiratory illness, recent influenza-like illness and various symptoms such as fever, sore throat and cough. Antibody concentrations were initially explored as a continuous exposure variable, then categorised into 'positive' (>12 U/ml), 'equivocal' (8-12 U/ml) and 'negative' (>8 U/ml) categories based on standard laboratory thresholds. Equivocal results were dropped for analyses.

6.4.8.iii Classification of confounding variables

Information on potential demographic and clinical confounding factors was generally collected as a categorical variable eg a question on personal history of hypertension could be answered using the following: 'yes on medication', 'yes but no medication', 'no', but categories combined into a binary 'yes/no' response for analyses. Age was grouped into 10-year age-groups from 40 years (the lower

limit for inclusion into the study) up to the age of ≥80 years. Ethnicity was grouped as 'Asian or Asian British' 'Black or Black British', 'Mixed', 'Other' and 'White' following Census classifications. Influenza vaccination status was divided into several categories: 'yes this year', 'yes last year, 'yes 2-5 years ago', 'yes >5 years ago' and 'no, never'. This was because participants may have had residual protection against seasonal influenza from influenza vaccinations received in previous years even if they had not received a vaccination this year. Vaccination status was then re-categorised as a binary variable 'yes this year' and 'no' (which included all other answers) for analyses: it was recognised that vaccinations from previous years would not protect against the circulating pandemic influenza strain. The variable BMI was derived from self-reported height and weight using the formula BMI = weight (kg) / height^2 (m) and grouped into <25 (normal), 25-29.9 (overweight), 30-39.9 (obese) and 40+ (morbidly obese) categories using standard thresholds.

6.4.9 Statistical analysis

Data were analysed using Stata (*Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP). X^2 tests were used to assess baseline comparability between cases and controls. Although numbers were relatively small, conditions for use of Fisher's exact test were not met[194]. Characteristics of participants with and without missing data were also compared using X^2 tests to assess any risk of bias associated with missing data. Information on potential confounding factors obtained from questionnaires was validated against information extracted from the medical notes on the same factors using Z tests for difference in proportions.

First I used univariable logistic regression analysis to investigate associations between recent influenza-like illness, acute respiratory illness or presence of influenza IgA antibodies and case/ control status. Here the log odds ratio for AMI in those exposed to recent influenza compared to those unexposed was calculated and p values were presented from the Wald test of the null hypothesis that the true parameter value of the log odds ratio = 0. The same approach was taken using influenza vaccination as the exposure variable to explore any protective effect against AMI.

Second I examined potential confounding factors for independent associations with both the exposure (influenza) and the outcome (AMI) using X^2 tests. Multivariable logistic regression models were then generated that included the main outcome and exposure as well as age-group, gender and timing of admission (factors on which frequency matching was done), influenza vaccination status (an a priori confounder) and other potential confounding factors. These were examined in a backwards stepwise procedure using likelihood ratio tests to test the effect of removing each one sequentially. Where p values from likelihood ratio tests were <0.1, the factor remained in models.

Third I explored potential interactions using Mantel Haenszel methods to generate stratum-specific odds ratios for the effect of influenza on AMI and to test for heterogeneity between strata. Where there was evidence of heterogeneity between strata, results were presented by stratum and an interaction term fitted into models. The effect of including an interaction term between factors compared to including factors separately in models was tested using the Wald test for interaction.

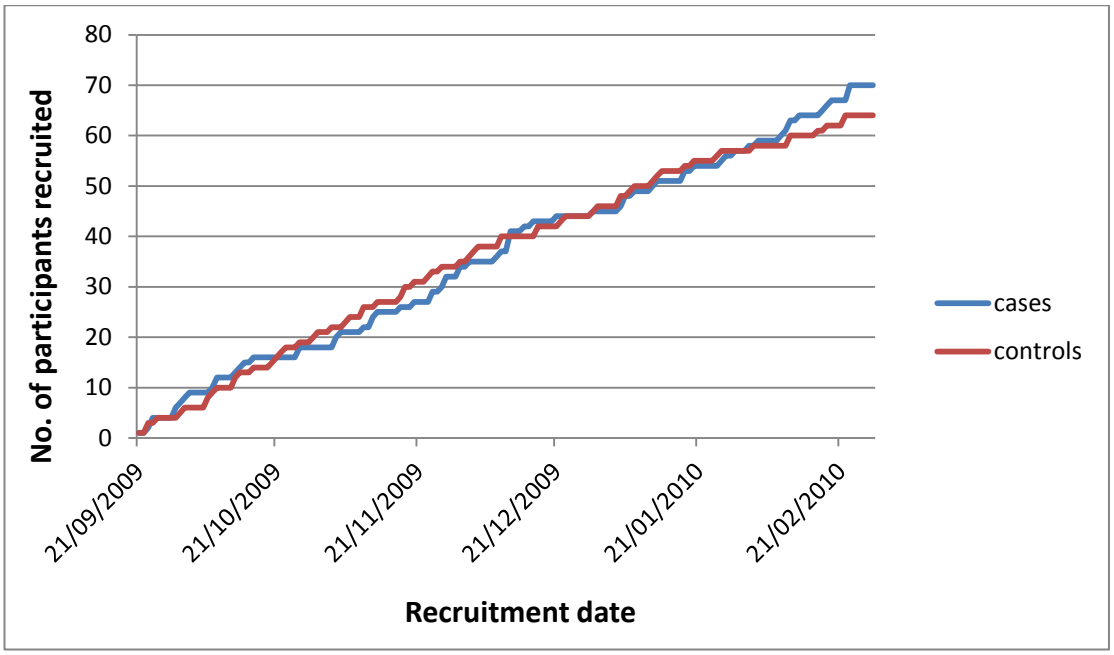
An interim analysis was planned after the first season of data collection to monitor recruitment, assess feasibility of data collection methods, investigate initial results and plan for a second season of data collection if necessary. In the event of under-recruitment, interim results would be used to infer the sample size needed for a full-scale study.

6.5 Results

6.5.1 Characteristics of participants

Between 21/09/2009 and 28/02/2010, 134 participants were recruited. These were 70 cases and 64 controls, for whom acceptance rates were 66% and 67% respectively (figure 6.1).

Figure 6.1 Cumulative frequency of participant recruitment over time



Median age was 63.6 years (IQR 53.3 - 72.6) and 21% of participants were female. Cases were significantly more likely to be of Asian or Asian British ethnicity (p=0.016), to have a previous history of myocardial infarction (p=0.04) and a family history of myocardial infarction (p<0.001) than controls (table 6.4). Of 70 patients hospitalised with AMI, 48 met criteria for STEMI, 17 had a NSTEMI and in 5 cases the subtype of myocardial infarction was unspecified. Control patients were admitted for a range of acute surgical problems that included colorectal, urological and orthopaedic conditions.

Characteristic	Cases (n=70)	Controls (n=64)	P value
Age group			
40-49	8 (11.4)	13 (20.3)	0.61
50-59	19 (27.1)	18 (28.1)	
60-69	19 (27.1)	15 (23.4)	
70-79	17 (24.3)	11 (17.2)	
80+	7 (10.0)	7 (10.9)	
Gender			
Female	13 (18.6)	15 (23.4)	0.49
Male	57 (81.4)	49 (76.6)	
Month of admission			
September	8 (11.4)	7 (10.9)	0.92
October	12 (17.1)	15 (23.4)	
November	15 (21.4)	14 (21.9)	
December	10 (14.3)	10 (15.6)	
January	14 (20.0)	11 (17.2)	
February	11 (15.7)	7 (10.9)	
Ethnicity			
Asian or Asian British	18 (25.7)	6 (9.4)	0.03*
Black or Black British	2 (2.9)	0 (0.0)	
Mixed	0 (0.0)	1 (1.6)	
White	50 (71.4)	57 (89.1)	
Smoker			
No never	22 (31.4)	23 (35.9)	0.77
Yes current	27 (38.6)	21 (32.8)	
Yes ex	21 (30.0)	20 (31.3)	
Diabetes			
No	56 (80.0)	52 (81.3)	0.86
Yes	14 (20.0)	12 (18.8)	
Hypertension			
No	33 (47.1)	38 (59.4)	0.16
Yes	37 (52.9)	26 (40.6)	
Hypercholesterolaemia			
No	36 (51.4)	36 (56.3)	0.58
Yes	34 (48.6)	28 (43.8)	
Personal history of AMI			
No	56 (80.0)	59 (92.2)	0.04
Yes	14 (20.0)	5 (7.8)	
Personal history of stroke			
No	69 (98.6)	60 (93.8)	0.14
Yes	1 (1.4)	4 (6.3)	
Family history of AMI			
No	27 (38.6)	45 (70.3)	<0.001
Yes	43 (61.4)	19 (29.7)	
Family history of stroke			
No	65 (92.9)	58 (90.6)	0.64
Yes	5 (7.1)	6 (9.4)	
BMI category			
18.5-24.9	20 (30.8)	23 (39.0)	0.41
25.0-29.9	36 (55.4)	24 (40.7)	
≥30.0	9 (13.8)	12 (20.3)	
Influenza vaccination status†			
Vaccinated	30 (42.9)	29 (45.3)	0.78
Unvaccinated	40 (57.1)	35 (54.7)	

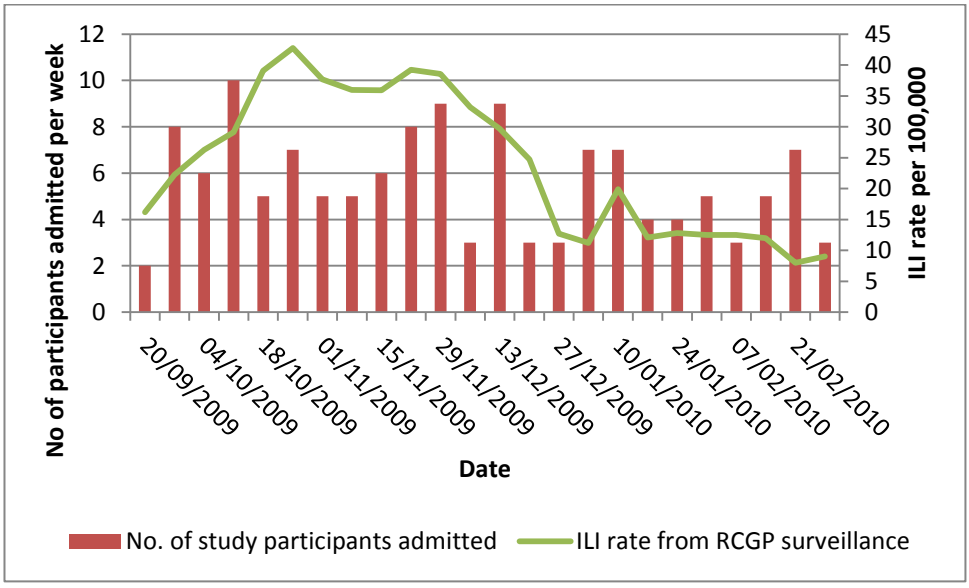
Table 6.4 Characteristics of study participants, n=134

*p=0.016 when comparing proportions who are of Asian or Asian British ethnicity to White ethnicity †'Vaccinated' refers to receiving influenza vaccination in the current vaccination year (ie since September 2009). All other years are classed as unvaccinated as the circulating pandemic strain was not covered by previous years' vaccines

6.5.2 Timing of participants' admissions in relation to national influenza circulation

A comparison of study participants' admission dates with rates of influenza-like illness in the community, based on GP consultations per 100,000 of the population is shown in figure 6.2. The peak week for ILI consultations in England & Wales was week 43 (ending 25th October 2009) where the rate was 42.8 per 100,000. This was also the peak week for influenza virus circulation according to data from virological sentinel surveillance schemes, when the proportion of positive specimens reached 41.2%. Our recruitment period spanned this period of peak influenza circulation.

Figure 6.2 Number of study participants admitted by influenza surveillance week compared to weekly ILI rates from national RCGP surveillance data



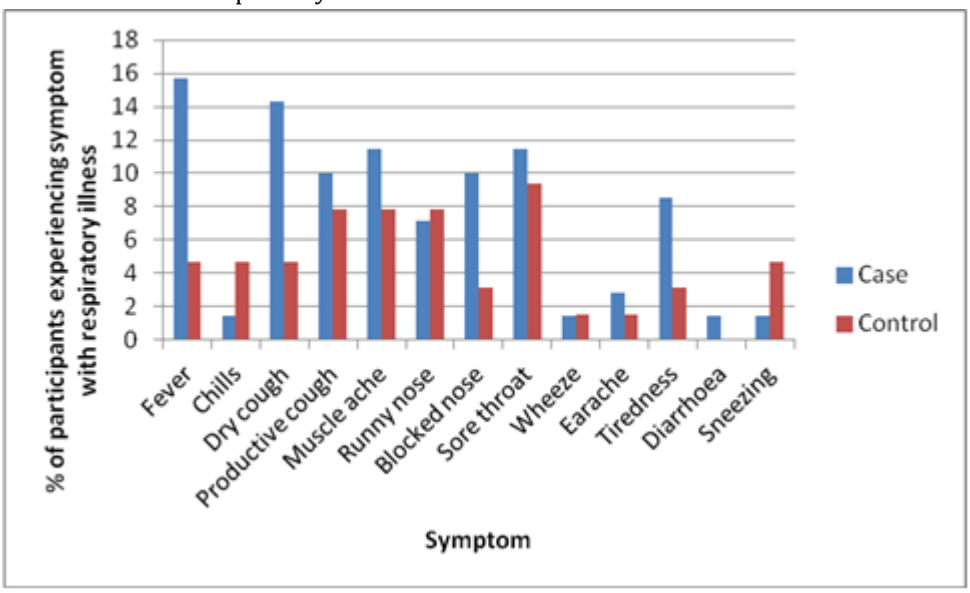
6.5.3 Description of exposures

6.5.3.i Recent respiratory illness

29 episodes of recent respiratory illness were reported by 17 cases (24.3%) and 12 controls (18.8%). 13 illnesses met criteria for influenza-like illness; these were reported by 10 cases (14.3%) and 3 controls (4.7%). The most frequently

reported time for the start of respiratory illness was 8-14 days before admission (31.0% of illnesses), and 4-7 days was the most frequently reported length of illness (37.9%). Symptom profiles of participants reporting recent respiratory illness are shown in figure 6.3. No swabs tested positive for influenza virus nucleic acid.

Figure 6.3 Percentage of cases (n=70) and controls (n=64) reporting various symptoms in the context of recent respiratory illness



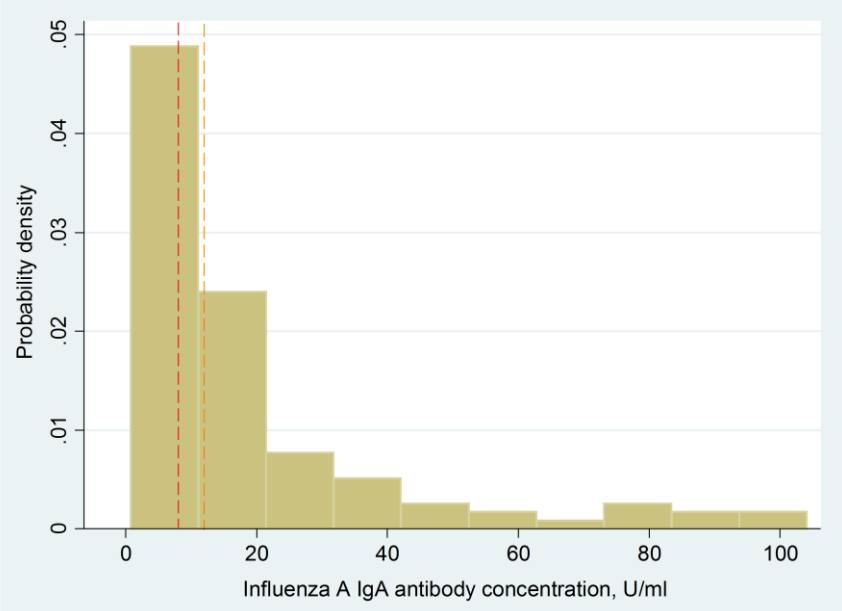
6.5.3.ii Laboratory results

Serum samples were available on 113 of 134 participants (84.3%). Characteristics of participants with and without missing serological data were similar and are shown in table 6.5. 53/113 (46.9%) of participants tested positive for serum influenza A IgA antibodies, representing 25 cases (43.1%) and 28 controls (50.9%). 62% of participants who were seropositive had received influenza vaccination compared to 31% of seronegative participants. A further 4 cases (7.3%) and 4 controls (6.9%) had an equivocal result despite repeat testing. Antibody titres ranged from 0.8-104.1 U/ml (median 10.8, IQR 4.8-21.0). The distribution of antibody titres is shown in figure 6.4.

Characteristic of participant	Laboratory data not missing, n=113(%)	Laboratory data missing, n=21 (%)	P value
Age-group			
40-49	18 (15.9)	3 (14.3)	0.70
50-59	30 (26.6)	7 (33.3)	
60-69	30 (26.6)	4 (19.0)	
70-79	22 (19.5)	6 (28.6)	
80+	13 (11.5)	1 (4.8)	
Gender			
Male	90 (79.7)	16 (76.2)	0.72
Female	23 (20.3)	5 (23.8)	
Smoker (questionnaire data)	38 (33.6)	7 (33.3)	0.66
No never	42 (37.2)	6 (28.6)	
Yes current	33 (29.2)	8 (38.1)	
Yes ex			
Diabetes			
No	92 (81.4)	16 (76.2)	0.58
Yes	21 (18.6)	5 (23.8)	
Hypertension			
No	58 (51.3)	13 (61.9)	0.37
Yes	55 (48.7)	8 (38.1)	
Hypercholesterolaemia			
No	59 (52.2)	13 (61.9)	0.41
Yes	54 (47.8)	8 (38.1)	
Personal history of AMI			
No	96 (85.0)	19 (90.5)	0.51
Yes	17 (15.0)	2 (9.5)	
Family history of AMI			
No	60 (53.1)	12 (57.1)	0.73
Yes	53 (46.9)	9 (42.9)	
'Case' status			
No	55 (48.7)	9 (42.9)	0.62
Yes	58 (51.3)	12 (57.1)	
Respiratory illness			
No	91 (80.5)	14 (66.7)	0.16
Yes	22 (19.5)	7 (33.3)	
Influenza-like illness			
No	103 (91.2)	18 (85.7)	0.44
Yes	10 (8.8)	3 (14.3)	
Fever	101 (89.4)	18 (85.7)	0.63
No	12 (10.6)	3 (14.3)	
Yes			
Influenza vaccination status			
Vaccinated	51 (45.1)	8 (38.1)	0.55
Unvaccinated	62 (54.9)	13 (61.9)	

Table 6.5 Characteristics of participants with and without missing laboratory data
NB Due to rounding, percentages may total >100.0%

Figure 6.4 Influenza A IgA antibody concentrations in serum samples, n=113



Note values to the left of the red dotted line denote a negative result, values between the red and orange dotted lines represent an equivocal result and values to the right of the orange dotted line show a positive result.

6.5.4 Associations between exposures and potential confounders

Table 6.6 shows p values from X^2 tests for the association between various exposures and potential confounders. Factors with a p value of <0.2 are highlighted as these were subsequently considered for inclusion in multivariable regression models (along with age-group, gender, timing of admission and influenza vaccination status).

6.5.5 Uni- and multivariable models from logistic regression analysis

Although differences were not statistically significant, cases were more likely to have reported recent influenza-like illness than controls – adjusted OR 3.17 (95% CI 0.61 - 16.47) as well as other key respiratory illness symptoms. There was a non-significant trend towards a protective effect of influenza vaccination against AMI – adjusted OR 0.46 (95% CI 0.19 - 1.12). Results from the logistic regression analysis are summarised in table 6.7.

Potential confounder	Exposure variable						
	Respiratory illness	**Influenza-like illness**	**Fever**	**Cough**	**Sore throat**	**Influenza IgA antibodies**	**Influenza vaccination**
Age-group	0.23	**0.18**	**0.09**	0.54	0.25	0.88	**0.001**
Gender	0.98	0.22	**0.15**	0.79	0.88	0.60	**0.06**
Month of admission	0.22	0.40	0.35	0.34	**0.17**	0.56	**<0.001**
Ethnicity	0.83	0.91	0.76	0.63	0.92	0.59	0.63
Smoker (questionnaire)	**0.09**	0.30	0.21	**0.003**	0.22	0.65	**0.16**
Smoker (medical records)	0.32	0.85	0.82	0.25	**0.18**	0.57	**0.04**
Diabetes	0.47	0.28	0.45	0.61	0.75	0.59	0.50
Hypertension	0.57	0.95	0.98	0.56	**0.08**	0.77	**0.03**
Hypercholesterolaemia	0.81	0.56	0.56	0.50	**0.03**	0.92	**0.05**
Personal history of AMI	0.26	**0.07**	**0.02**	**0.03**	0.26	**0.11**	**0.02**
Personal history of stroke	0.23	0.46	0.42	0.21	0.37	**0.08**	0.46
Family history of AMI	0.28	**0.08**	**0.09**	**0.13**	0.40	0.77	0.81
Family history of stroke	0.64	0.32	**0.04**	0.28	0.63	**0.08**	0.92
BMI category	0.25	0.28	0.26	0.47	0.89	0.20	0.64
Influenza vaccination status	0.92	0.87	0.83	0.58	0.58	**0.001**	**-**

Table 6.6 p values from X^2 tests of the association between respiratory illness exposures and potential confounders

Factors with p values <0.2 are highlighted in bold. These factors were considered for inclusion in multivariable models, along with age group, gender, month of admission (the variables used for frequency matching) and influenza vaccination status (an a priori confounder)

Exposure variable	Prevalence – cases, n(%)	Prevalence – controls, n(%)	Unadjusted odds ratio (95% CI)	Adjusted odds ratio* (95% CI)
1. Respiratory illness	17 (24.3)	12 (18.8)	1.39 (0.60-3.19)	1.39 (0.56-3.47)
2. Influenza-like illness	10 (14.3)	3 (4.7)	3.39 (0.89-12.92)	3.17 (0.61-16.47)
3. Fever	11 (15.7)	4 (6.3)	2.80 (0.84-9.28)	2.42 (0.54-10.98)
4. Cough	21 (30.0)	10 (15.6)	2.31 (0.99-5.40)	2.04 (0.76-5.47)
5. Sore throat	10 (14.3)	8 (12.5)	1.17 (0.43-3.17)	1.43 (0.44-4.69)
6. Influenza A IgA antibodies±	25 (46.3)	28 (54.9)	0.71 (0.33-1.53)	0.82 (0.34-2.00)

Table 6.7 ORs for the association between AMI and respiratory illness exposures, unadjusted and adjusted

*Adjustments were made for age-group, gender, month of admission and influenza vaccination status (all exposures), family history of myocardial infarction (exposures 2, 3, 4 & 5) and personal history of myocardial infarction (exposures 2, 3,4 & 5)

±Note that n=105 (54 cases and 51 controls) for influenza antibodies where equivocal results are excluded, compared to n=134 (70 cases and 64 controls) for all other exposures

Although the effect of interaction terms was examined (data not shown) no interaction terms were fitted in final models. It was recognised that statistical tests for heterogeneity between strata were likely to be underpowered to detect an effect in this analysis.

6.5.6 Validation of questionnaire information against medical records

Comparing information on several potential confounding factors – personal history of AMI, diabetes, hypertension, hypercholesterolaemia and smoking status – from questionnaires and medical records revealed very similar results for all except smoking status (table 6.8). While 97.7% of those reporting themselves to be non-smokers had a non-smoking history in the medical notes, 45.6% of people described themselves as 'ex smokers' when the medical notes identified them as 'current smokers' and only 54.3% had a current smoking history according to both questionnaires and medical record. Due to this discrepancy, both smoking self-report and history from medical records were tested separately for inclusion in models.

Variable	Prevalence from questionnaire (%)	Prevalence from record (%)	P value
Smoker			
No never	45 (33.6)	43 (33.9)	<0.001
Yes current	48 (35.8)	81 (63.8)	
Yes ex	41 (30.6)	3 (2.4)	
Diabetes			
No	108 (80.6)	104 (80.6)	0.99
Yes	26 (19.4)	25 (19.4)	
Hypertension			
No	71 (53.0)	68 (54.0)	0.87
Yes	63 (47.0)	58 (46.0)	
Hypercholesterolaemia			
No	72 (53.7)	68 (54.0)	0.97
Yes	62 (46.3)	58 (46.0)	
Personal history of AMI			
No	115 (85.8)	111(86.7)	0.83
Yes	19 (14.2)	17 (13.3)	
Personal history of stroke	129 (96.3)	121 (94.5)	0.50
No	5 (3.7)	7 (5.5)	
Yes			

Table 6.8 Comparison of information obtained from questionnaires and medical records

6.5.7 Inference of sample size needed based on season 1 of recruitment

The proportion of controls exposed to recent influenza-like illness was 4.7% with an adjusted odds ratio of 3.17. Using the Fleiss method the sample size needed was 222 cases and 222 controls to detect a result with 90% power at the 5% significance level (or 245 of each with a continuity correction). Though high, the expected odds ratio was in keeping with incidence ratios generated through self-controlled case series analysis in chapters 4 and 5. Doing a similar calculation using the exposure respiratory illness, the proportion of exposed controls was 18.8%. With an adjusted odds ratio of 1.39 this resulted in a sample size requirement of 1154 cases and 1154 controls (or 1190 of each with a continuity correction).

6.5.8 Decisions regarding further recruitment

For reasons outside the scope of the project (maternity leave) it was not possible to recruit participants during the 2010/11 influenza season. A decision was taken following the PhD upgrade meeting and viva on 28th March 2011 to cease recruitment after one season, rather than to conduct the study during the 2011/12 season. This was because, using results from the corrected sample size calculations informed by season one, even at the lowest level 222 cases and controls would be needed (total 444 participants) for the study to be adequately powered. Despite relatively high uptake, only 134 participants were recruited in the first season. Without additional resources it was not possible that another 310 participants could be recruited in a second season, especially as the unpredictable nature of influenza circulation meant that seasons could last for fewer months than expected. During the 2009 influenza pandemic, there was a mismatch between age-groups infected by H1N1pdm09 and those typically affected by AMI which further reduced the study's power to detect an effect.

6.6 Discussion

6.6.1 Summary of findings

The study was supportive of the hypothesis that recent respiratory illness and in particular influenza-like illnesses occurring during the second wave of the 2009 influenza pandemic were more common in patients hospitalised with AMI than with acute surgical conditions, although differences were not statistically significant. There was a (non-significant) trend towards protection against AMI with influenza vaccination. Based on current ILI rates it is estimated that at least 222 cases and 222 controls would be needed for a full scale case control study.

6.6.2 Study strengths

Triangulating data on influenza from several sources including symptom report, virus detection and antibody testing, allowed sensitivity analyses to be done using different influenza definitions. The accuracy of information on admission diagnosis and potential medical confounding factors obtained through self-report was also confirmed using medical records. The study proved successful at demonstrating the feasibility of recruitment from hospital wards, the acceptability of the study to patients and the effectiveness of questionnaire and database design.

6.6.3 Study limitations – lack of power and missing data

While I had hypothesised that more adults would be infected during the second pandemic wave due to the expected upwards shift in age distribution of infections, national ILI rates remained low throughout this period so the study was underpowered to detect an effect. In addition some variables were affected by missing data: only 113 participants (84.3%) had a usable serum sample and only 105 (78.4%) samples had a definite positive or negative result on IgA ELISA. Some samples were missing due to patients being missed on the phlebotomist's round and others due to difficulties obtaining a blood sample. As there were no

substantial differences in characteristics of participants with and without missing laboratory data, however, this is unlikely to have significantly biased results.

6.6.4 Study limitations – misclassification of exposure

Using self-reported recent respiratory and influenza-like illness as exposures introduced the possibility of reporting or recall bias. Nevertheless this method allows greater sensitivity to detect recent respiratory symptoms than relying on reports of medically attended illnesses, which comprise only a small minority of influenza cases[195]. As cases and controls were frequency matched on week of admission, external factors such as media coverage of the influenza pandemic should not have had a differential effect on respiratory illness reporting. It was perhaps unsurprising that none of the nasopharyngeal and throat swabs was positive for influenza virus given a) the low rates of infection in this age-group[175] and b) that the majority of viral shedding in influenza occurs in the first 2-3 days after symptom onset[19] and most reported respiratory symptoms in study participants occurred 8-14 days before admission.

Influenza serology is difficult to interpret in vaccinated participants as it not possible to distinguish antibody rises caused by infection from those caused by vaccination. Validation of the IgA assay used suggests it has acceptable sensitivity and specificity to detect recent seasonal influenza A infection[191] but its effect with H1N1pdm09 is unclear. It has previously been noted that serological studies carried out during the pandemic were severely hampered by cross reactivity both with vaccine and with seasonal influenza strains[177]. It would have been useful to have validated the IgA ELISA against results obtained from the more widely used haemagglutinin inhibition (HI) assay for IgG on paired samples, which uses the more standard technique of serial dilutions. This was not done for several reasons: HI assays require several controls for standardisation and are time-consuming and labour-intensive[188]; participants were not due to attend for routine follow-up during the time window in which a second blood sample was needed; in people with recent (rather than current) influenza it was likely that an antibody response would already be maximal on the first test, limiting the usefulness of a second sample.

6.6.5 Logistic issues and lessons learned

From a training perspective, conducting research in a busy acute setting, developing clinical and laboratory collaborations, and adapting the study in response to changing external factors (such as the advent of the 2009 influenza pandemic) were all valuable lessons learned. In future a similar study would be improved by having a recruitment team able to operate across multiple sites to maximise recruitment during the influenza season. This would reduce the impact of losing potential participants before recruitment due to transfers to other hospitals or problems fitting recruitment around clinical care. Having a dedicated study phlebotomist would have increased the number of samples obtained, although part of the rationale for using ward phlebotomists to take study blood samples at the same time as routine samples was to minimise inconvenience and the number of tests that patients required.

The unexpected coincidence of the study start date with the 2009 influenza pandemic led to several issues with study design and communication. During the pandemic, the hospital infection control team followed up any in-patient on whom a nasopharyngeal swab was taken for influenza testing. When this came to light I reported details of all study participants to the team on a daily basis to prevent these patients from being following up as potential influenza cases. For the first time, in the autumn and winter of 2009/2010 patients attending the GP for influenza vaccination were offered both seasonal and pandemic influenza vaccinations. In the questionnaire when people said that they had been vaccinated 'this year' I did not know (and it seemed that many participants did not know) which vaccination they had had. Therefore some participants labelled as 'vaccinated' may in fact have had only seasonal influenza vaccine based on the previous year's circulating strains which would not have provided protection against pandemic strain influenza. This highlights the need to pilot data collection systems and to evaluate them early in the study to incorporate changes.

6.6.6 Interpretation of results in context of previous studies

There are no previous individual-level studies of the association between H1N1pdm09 and AMI that met inclusion criteria for the systematic review update (appendix to chapter 2). The low rates of ILI and acute respiratory illness seen in hospitalised participants in our study (whose median age was 63.6 years) were in keeping with other studies of H1N1pdm09 suggesting that most illnesses were mild and typically affected younger people[176]. There have been four small case control studies examining the association between clinically-defined ILI in AMI patients during periods of seasonal influenza circulation[86–89]. Two of these studies found a significant association between AMI and recent ILI[86,89]. In the other two studies, the point estimate tended towards an effect but results failed to reach statistical significance[87,88]. Our results are consistent with these studies. Though findings should be interpreted with caution, this study supports the hypothesis that, as with other influenza strains, H1N1pdm09 could trigger AMI in vulnerable groups. Nonetheless the population impact of H1N1pdm09 on rates of AMI hospitalisations and death is likely to have been relatively low given the mismatch between the ages of those typically affected by H1N1pdm09 and AMI as well as the relatively mild clinical effects of this strain.

6.6.7 Future directions

Studies described in this thesis have used varying clinical and laboratory definitions of influenza to investigate the strength, temporality, gradient and consistency across time periods and strains of an association between influenza and AMI. Some vulnerable populations have been identified. So far, though, the biological plausibility of an effect has not been investigated. In chapter 7 I discuss potential biological mechanisms through which influenza could act to trigger AMI and describe an exploratory study of inflammatory and haemostatic markers.

SUMMARY

- A case control study was used to investigate whether patients hospitalised for AMI were more likely to have experienced recent ILI during the second wave of the 2009 influenza A H1N1 pandemic than patients hospitalised for a range of acute non-vascular surgical conditions
- Of 134 participants, 29 (21.6%) reported recent respiratory illness of whom 13 (9.7%) had illnesses meeting ILI criteria
- AMI cases were more likely to have reported ILI than controls – adjusted OR 3.17 (95% CI 0.64 - 16.76) – as well as other key respiratory symptoms but differences were not statistically significant.
- Compared to seasonal influenza, during the pandemic the age groups typically affected by AMI had comparatively low rates of influenza infection which decreased the study's power to detect an effect

7. Inflammatory and haemostatic mechanisms through which influenza could trigger acute myocardial infarction

7.1 Description of chapter contents

The first part of this chapter describes inflammatory and haemostatic mechanisms associated with influenza and AMI. This provides a rationale and theoretical framework to underpin the exploratory study described in the second half of the chapter. Section 7.2 describes acute inflammatory and haemostatic response to influenza and other tissue insults. Section 7.3 outlines the roles of inflammation and haemostasis in atherosclerosis and AMI. Section 7.4 discusses theoretical mechanisms for an interaction between the acute phase response to influenza and the triggering of acute cardiovascular events. In section 7.5, several prominent markers of inflammation and haemostasis relevant to both influenza and AMI are discussed in detail. I then describe an exploratory mechanistic study in sections 7.6-7.9. Here, AMI patients admitted to the Royal Free Hospital (who comprise the 'cases' in the case control study described in chapter 6) have extra blood samples taken and tested for a range of cytokines and inflammatory markers. Comparisons are made between levels of biomarkers in AMI patients with and without evidence of recent influenza infection.

7.2 Inflammatory and haemostatic responses to acute tissue insults

7.2.1 Acute phase inflammatory response to influenza

While the innate immune response to influenza comprises a range of rapid co-ordinated defence mechanisms including physical defences (eg mucus and cilia), interferons and complement, this section will focus on one of these – the acute inflammatory response. An acute phase response is triggered by influenza virus as well as a range of tissue insults such as other infections or ischaemic injury. It is characterised by rapid rises in local pulmonary levels of inflammatory cytokines and chemokines[196]. Cytokines such as tumour necrosis factor-α (TNF-α), interleukin -6 (IL-6) and IL-18 and chemokines such as RANTES, MIP-1a, monocyte chemoattractant protein-1 (MCP-1) and MCP-3 are produced from

influenza virus-infected monocytes and macrophages and, to a lesser extent, virus-infected respiratory epithelial cells[197]. These pleiotropic agents mediate a variety of inflammatory processes in different tissues, for example acting on the brain to induce fever[198], the liver to stimulate the secretion of acute phase proteins such as C-reactive protein[199], and on vascular endothelium to increase permeability and allow leukocyte extravasation and migration to the site of inflammation[200]. A graphical representation of this process is shown in figure 7.1[201].

The resulting inflammatory milieu favours development of antiviral and T helper cell type 1 immune responses by the adaptive immune system designed to combat influenza, for example through direct attack on antigen-bearing cells by cytotoxic T cells[202]. Humoral (or antibody-mediated) immunity - another major component of the adaptive immune response to influenza - is described briefly in chapter 6, section 6.2 in relation to the research study described. Adaptive immunity will not be discussed further here.

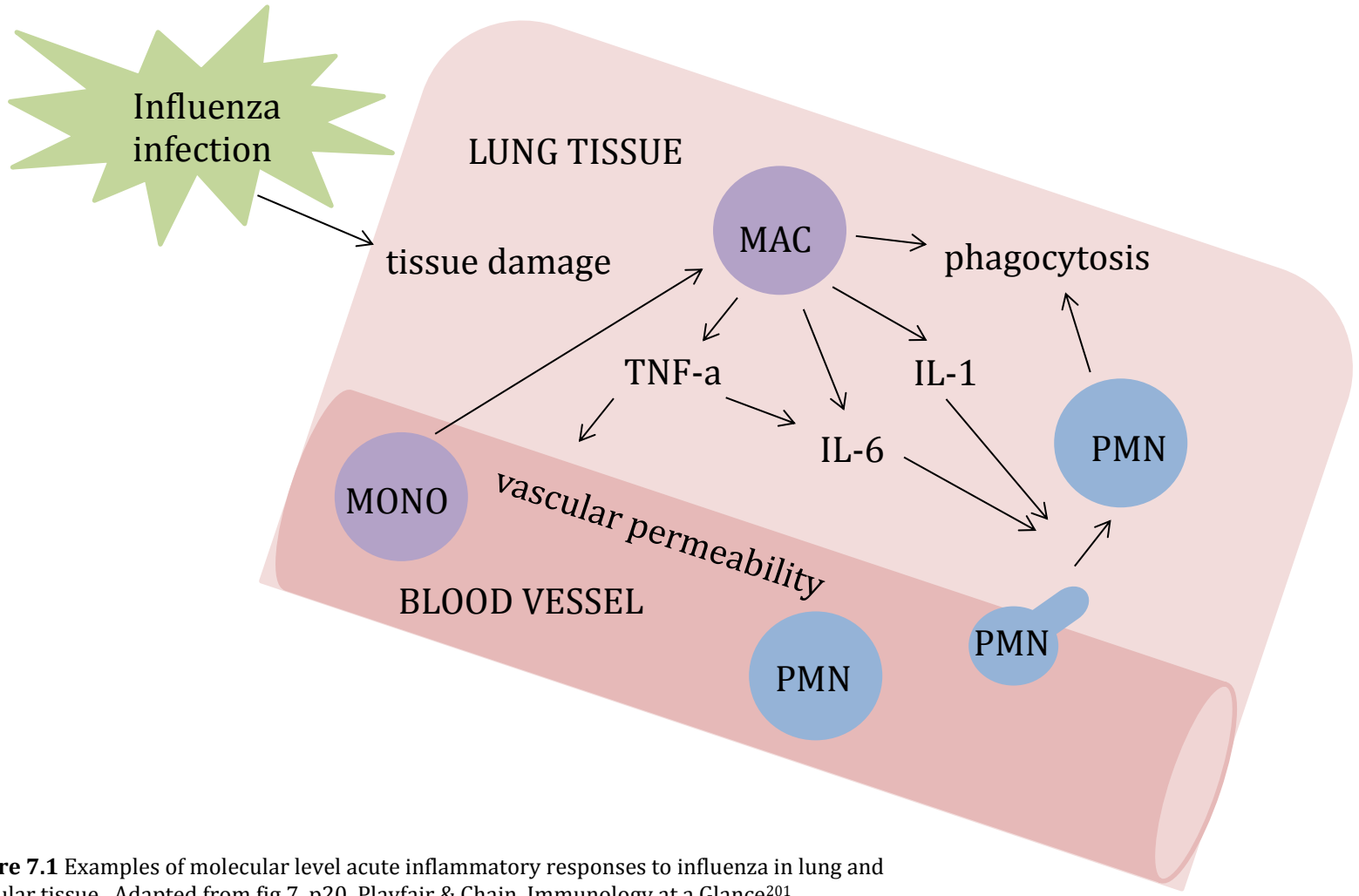


Figure 7.1 Examples of molecular level acute inflammatory responses to influenza in lung and vascular tissue. Adapted from fig 7, p20, Playfair & Chain, Immunology at a Glance[201]

PMN = polymorphonuclear leukocyte
MAC = macrophage
MONO = monocyte
TNF-α = tumour necrosis factor-α
IL-6 = interleukin-6

7.2.2 Haemostasis after tissue injury

Inflammatory and coagulation pathways are closely linked through a complex system of bi-directional crosstalk[203,204]. Both pathways are triggered by the same types of stimuli such as vascular injury or infection and their effects act in concert in the same tissues and disease states[205]. Blood coagulation, or haemostasis, is an intricate process involving platelet activation and aggregation, as well as a cascade of proteolytic reactions whereby circulating precursor proteins are cleaved to active products, leading ultimately to thrombin-mediated conversion of fibrinogen to a fibrin network[206] (figure 7.2). Key players are the endothelium, platelets, coagulation factors and inhibitors and the fibrinolytic system[206]. Inflammatory molecules and products interact with the coagulation pathway at every stage eg expression of the coagulation factor tissue factor is mostly dependent on the pro-inflammatory cytokine IL-6[204]; another prominent coagulation factor, thrombin, has pleiotropic anti-inflammatory actions including diminishing the release of IL-12 and favouring transformation of protein C to activated protein C (a potent inflammatory and anticoagulant molecule)[207]; a third important coagulation factor, fibrinogen, is considered to be an acute phase reactant[205] and is genetically correlated with C-reactive protein levels via links on chromosomes 12 and 21[208]. Powerful anti-coagulation systems regulate not only coagulation reactions but also inflammation[204]. Finally various coagulation factors including tissue factor, thrombin and fibrinogen have been significantly implicated in diseases with an inflammatory component, such as atherosclerosis[205]. This is discussed further in section 7.3.

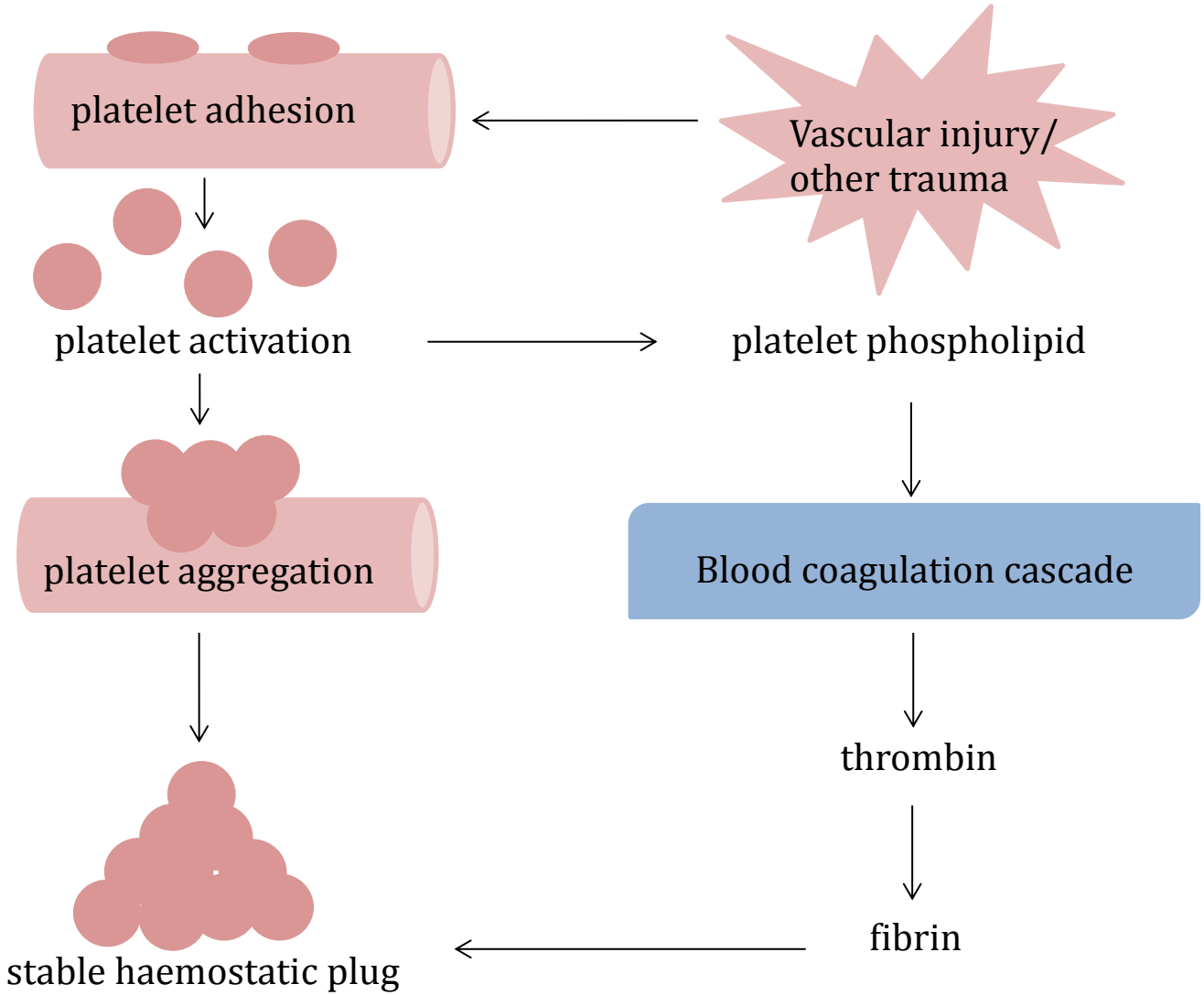


Figure 7.2 Platelet and haemostatic responses to vascular injury or trauma. Adapted from fig 22.1, p164, chapter 22, Hoffbrand, Essential Haematology[206]

7.2.3 Detecting inflammatory and haemostatic products after influenza infection or vaccination in vivo

It is clear that influenza infection stimulates expression or release of a wide variety of inflammatory molecules and cytokines (many of which are key mediators of atherosclerosis[209] as well as interacting with coagulation pathways. Various studies have examined products of inflammation and haemostasis after influenza infection, or influenza vaccination as an inflammatory stimulus. For such markers to be useful tools for epidemiological research, they must not only be expressed in circulating blood in response to influenza infection, but should have a validated, commercially available assay that is stable over time and to freeze-thaw cycles. Tables 7.1 and 7.2 below are not exhaustive but give examples of the types and temporal profiles of molecules that are expressed and measurable after influenza. Six important markers identified from these studies – C reactive protein, serum amyloid A, interleukin-6, tumour necrosis factor-α, P-selectin and von Willebrand factor – are measured in the exploratory study described later in this chapter. Further details on these individual markers are given in section 7.5.

SUMMARY 1)

- Influenza infection elicits acute local and systemic inflammation and haemostasis
- There is extensive crosstalk between inflammatory and haemostatic pathways
- Key markers of inflammation (such as CRP and SAA), cytokines (such as IL-6 and TNF-α) and markers of platelet activation (such as P-selectin) are increased in influenza and may be useful in mechanistic studies

Author (year)	Inflammatory stimulus	Methods	Results
Lannegard (2003)[210]	Various bacterial and viral infections in 98 patients (11 had influenza A)	Serum SAA and CRP levels measured in all patients on admission and correlated with each other	- Positive correlations between CRP and SAA found in both viral and bacterial infections - Mean CRP concentration = 85 in influenza patients - Median SAA = 980 mg/L in influenza patients
Lee (2007)[211]	Severe influenza A virus infection in 39 hospitalised patients	Acute and convalescent blood samples taken on admission and >10 days later and tested for a panel of 11 cytokines and chemokines	- Significant increases in IL-6, IL-8, IFN-induced protein 10 and monokine induced by IFN-gamma seen in acute phase. - RANTES increased in convalescent sample only - No change in IFN-gamma, IL-12, TNF- α , IL-10, IL-1B and MCP-1
Keller (2007)[212]	ILI in 54 subjects (9 had confirmed influenza)	Blood taken at baseline (pre-illness) then within 1-3 days of illness and 14 days later. Haemostatic proteins measured at each time-point	- VWF increased in the acute phase - Fibrinolysis was activated (measured by increased PAP) - There was no change in plasminogen activator inhibitor or prothrombin fragments
Kreutz (2007)[213]	Acute viral upper respiratory tract infection in 18 subjects compared to 8 healthy controls	Blood samples taken at enrolment and 6 weeks later for ADP-induced platelet aggregation and platelet surface receptor expression (P-selectin and glycoprotein IIb/IIIa) as well as inflammatory markers	- Platelet reactivity and P-selectin expression were higher during viral infection than in controls. Both fell significantly over time - CRP and TNF- α increased during viral infection - No difference in soluble P-selectin, sICAM-1 and sVCAM-1 levels in illness compared to recovery
Marchesi (2005)[214]	Serologically confirmed symptomatic influenza infection in 10 otherwise healthy volunteers	Endothelial function measured by brachial flow-mediated vasodilation (FMV), soluble ICAM-1 & VCAM-1. Inflammatory markers and lipid profile taken at 3 days and 3 months	- After 3 months, FMV was increased, compared to the measurement taken during infection - CRP, white cell count and HDL cholesterol were significantly lower at 3 months than during infection - CRP changes were correlated with sICAM-1 and sVCAM-1 changes
Nakayama (1993)[199]	A range of confirmed viral and bacterial infections in 288 hospitalised paediatric patients (25 had influenza)	SAA and CRP measured on day 1 (admission) and day 7 (convalescence)	- SAA and CRP higher on D1 than D7 in influenza-infected patients - Arithmetic mean SAA concentration = 63 mg/L in influenza - Arithmetic mean CRP concentration = <7 mg/L in influenza
Patel (2009)[215]	326 virus-positive URI episodes in 151 children aged 6-36 months (27 had influenza)	Children healthy at enrolment, followed for 1 year. Seen asap after each URI then again at day 3-7. Nasopharyngeal samples taken at first visit for cytokine analysis	- IL-1B, IL-6 and TNF- α were all raised and correlated with each other - IL-6 concentrations were significantly higher during influenza - IL-1B higher during URI episodes associated with acute otitis media than other episodes

Van Wissen[216] (2011)	ILI in 15 subjects (6 had confirmed influenza)	Blood taken at baseline (pre-illness) then within 1 day of illness, 2-3 days later and 14 days later. Haemostatic proteins measured at each time-point	- Large acute rise in hs_CRP, PAP, VWF and D-dimer cf baseline - No change in plasminogen activator inhibitor or prothrombin fragments - Other parameters of thrombin generation showed significant procoagulant change during infection and in the convalescent phase

Table 7.1 Studies of inflammatory and haemostatic markers after influenza or ARI

Author (year)	Inflammatory stimulus	Methods	Results
Carty (2006)[196]	Influenza vaccination given to 107 men (43 with severe carotid artery disease)	24 hour levels of cytokines measured	- CRP, Il-6 and SAA relatively higher in men with carotid artery disease (but difference only significant for SAA)
Lanza (2011)[217]	Influenza vaccination given to 28 patients with diabetes	Inflammatory and haemostatic markers were measured pre and post vaccine. 24 hour ECG and heart rate variability were assessed	- IL-6, CRP, platelet-monocyte aggregates and platelet-monocyte receptor expression were increased after vaccination - Heart rate variability decreased after vaccination
Liuba (2007)[218]	Influenza vaccination given to 8 healthy volunteers	Brachial artery responses to hyperaemia and sublingual GTN measured and carotid intima-media thickness assessed by external ultrasound before, 2 and 14 days after vaccination. Plasma CRP, fibrinogen, cGMP and antibodies against oxidised LDL measured	- CRP and fibrinogen elevated slightly on D2 resolved by D14 - oxLDL antibody levels increased above baseline D2 and D14 - Flow mediated dilatation of brachial artery decreased at D2 with a further decrease at D14. - No change in dilatory responses to GTN or carotid intima-medial thickness seen
Tsai (2005)[219]	Influenza vaccination given to 22 healthy individuals	Plasma CRP, IL-6, MCP-1, TNF-α, IL-2 soluble receptor-α and SAA measured pre vaccine and 1,3 and 7 days afterwards	- hsCRP, IL-6 and SAA all mildly increased on D1 and hsCRP on D3 - Plasma triglyceride levels decreased n D1, 3 and 7 - No change to MCP-1, TNF-α or IL-2 soluble receptor-α
Vlachopoulos (2011)[220]	Influenza vaccination or placebo given to 24 HIV-infected patients in an RCT	Endothelial function measured by flow-mediated dilatation (FMD) and inflammatory markers assessed before and at 8 and 48 hours post vaccine	- FMD deteriorated after vaccination at 8 and 48 hours - White blood cell count was elevated at 8 and 48 hours - Soluble ICAM-1 decreased maximally at 48 hours - There were no changes in endothelial function or inflammatory markers in the placebo group
Werba (2008)[221]	60 patients received influenza vaccine (26 with quiescent CHD and 34 with previous ACS)	Blood taken at baseline and 48 hours after vaccination for CRP and SAA	- CRP increased significantly in both groups - SAA increased significantly only in the group with previous ACS

Table 7.2 Studies of inflammatory and haemostatic markers after influenza vaccination as an inflammatory stimulus

7.3 Pathogenesis of atherosclerosis and AMI

7.3.1 Development of atherosclerosis

Atherosclerosis is a dynamic inflammatory disease, with inflammation central to all stages of its pathogenesis from inception and development of atherosclerotic lesions to end-stage thrombotic complications[6]. In its earliest stages, endothelial dysfunction is mediated through attachment of leukocytes to the vascular cell wall by molecules such as vascular cell adhesion molecule-1 and members of the selectin family[222]. Expression of cell adhesion molecules on vascular endothelium may be initiated by the action of cardiovascular risk factors such as smoking, hypertension and a high saturated fat diet[6]. After adhesion, chemokines such as monocyte chemoattractant protein-1 direct migration of monocytes into the artery wall, where they differentiate into macrophages and proliferate, under the influence of factors such as macrophage colony-stimulating factor[223]. Numerous coagulation factors have also been implicated in these processes[207]. Activated macrophages engulf modified lipoprotein particles by endocytosis[224]. Macrophages accumulate intra-cytoplasmic droplets of cholesterol ester and become known as 'foam cells'. These form early atherosclerotic lesions known as fatty streaks[52] (figure 7.3). With continued cycles of inflammation, accumulation of leukocytes, migration and proliferation of smooth muscle cells and fibrosis, lesions enlarge and remodel[7]. Complicated atherosclerotic plaques comprise a thrombogenic lipid core overlain by a fibrous cap[7], which may protrude into the vessel lumen and affect blood flow (figure 7.4).

Figure 7.3 Formation of a fatty streak
Figure designed by C. Warren-Gash

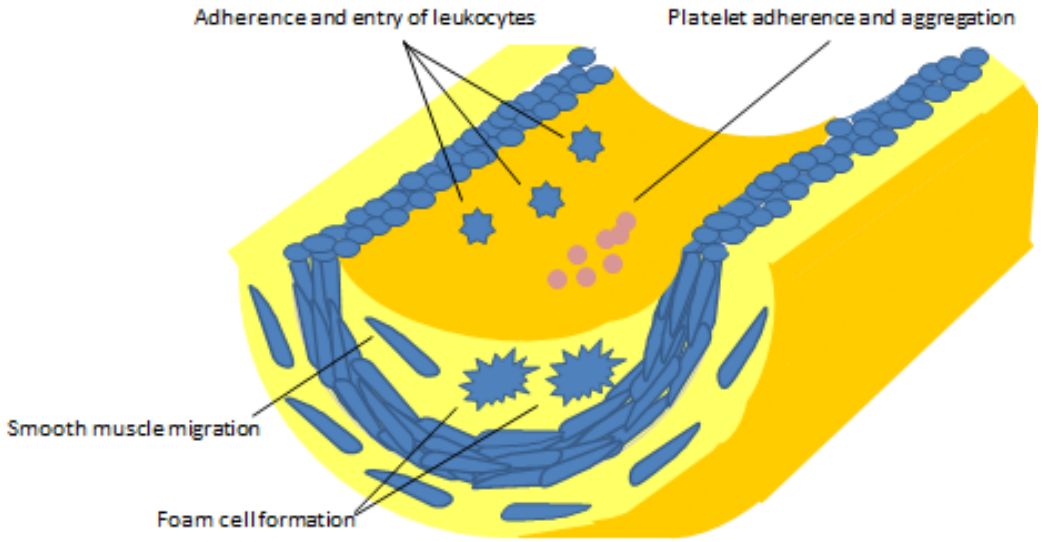
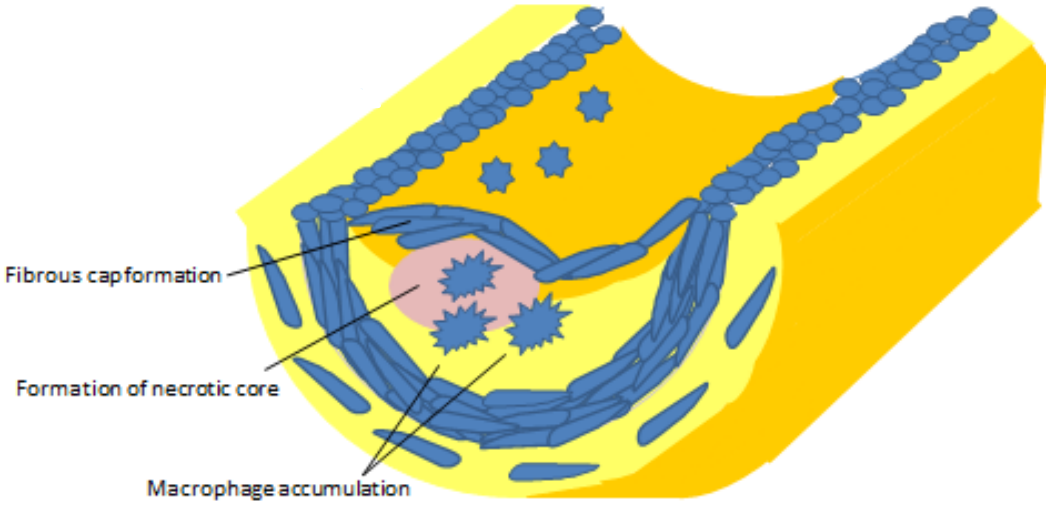


Figure 7.4 Formation of a complicated atherosclerotic plaque
Figure designed by C. Warren-Gash



7.3.2 Inflammation and haemostasis in AMI

Coronary artery thrombosis leading to AMI usually results from plaque rupture, rather than a critical artery narrowing[225]. The transition from stable to unstable (and ruptured) plaque involves disruption of the integrity of interstitial collagen matrix forming the plaque's fibrous cap[226]. This is mediated by fibrinolytic enzymes such as matrix metalloproteinases[226], which are expressed by macrophages in response to stimulation by pro-inflammatory cytokines. These cytokines also act on smooth muscle to prevent formation of new collagen fibres[6]. Platelets and the coagulation system are activated by the damaged vessel wall leading to thrombus formation[227]. In addition, activated T lymphocytes can contribute to arterial thrombosis by producing CD-40L which stimulates macrophages to produce the highly thrombotic protein tissue factor[228]. Markers from the haemostatic pathway such as D-dimer and von Willebrand factor have an independent association with myocardial infarction risk after adjusting for established risk factor and inflammatory markers[227]. Similarly inflammatory markers such as CRP are associated with myocardial infarction risk independent of other established risk factor and haemostatic factors[229]. It is evident that both inflammation and haemostasis contribute to the final common pathway towards coronary artery thrombosis and AMI. This is reflected in the use of agents such as aspirin, which have pleiotropic actions including anti-platelet and anti-inflammatory properties[207], for both primary and secondary AMI prevention.

SUMMARY 2)

- Inflammation is central to the genesis of atherosclerotic plaques
- Pro-inflammatory and pro-thrombotic molecules are instrumental in the transition from stable to unstable atherosclerotic disease
- Markers of systemic inflammation (such as CRP) and haemostasis (such as Von Willebrand Factor) have been shown to predict AMI risk independent of other established risk factors

7.4 Possible mechanisms through which influenza could trigger AMI

7.4.1 Direct effects on coronary vessels

Influenza and other acute respiratory infections exert both direct local effects on coronary arteries and indirect systemic effects through which they might act to trigger AMI[109]. Direct infiltration of influenza virus RNA has been found in human atherosclerotic plaques, although the clinical significance of this is unknown[230]. Influenza may also lead to focal inflammatory changes within atherosclerotic plaques and coronary vessels[231,232]. This has been demonstrated in a mouse model of atherosclerosis in which 24 apolipoprotein E-deficient (ApoE $^{-/-}$) mice were injected with a lethal dose of influenza[233]. Histological aortic specimens from infected ApoE $^{-/-}$ mice showed increased intimal cellularity compared to non-infected ApoE $^{-/-}$ mice. 10 infected ApoE $^{-/-}$ mice also had a significant sub-endothelial infiltrate of smooth muscle cells, lymphocytes and macrophages and one had a sub-occlusive platelet and fibrin-rich thrombus. As well as stimulating plaque inflammation, it is proposed that the host immune response to infection may affect plaque composition and therefore vulnerability to rupture through mechanisms such as macrophage activation[209].

7.4.2 Indirect systemic effects

Influenza infection may also generate systemic inflammation, acting through some of the inflammatory mediators described above. There is increasing evidence that markers of systemic inflammation independently predict vascular risk[234]. People with systemic inflammatory disorders such as rheumatoid arthritis are at higher risk of AMI and other acute cardiovascular events[235]. Acting in concert with systemic inflammation, haemostatic effects of influenza virus including endothelial dysfunction[214], increased platelet reactivity and aggregation[200], increased plasma viscosity and formation of thrombi [204] can also predispose to AMI. Haemodynamic effects associated with infections include increased sympathetic activity, vasoconstrictive effects and changes in circulating blood volume[55]. These can increase biomechanical stress on coronary plaques, potentially triggering their disruption[56]. Immobility associated with bed rest as

well as dehydration might accompany influenza infection and potentiate these processes.

SUMMARY 3)

- Influenza might trigger AMI either through direct action on coronary vessels or through indirect systemic inflammatory, haemostatic or haemodynamic effects

7.5 Role of specific markers of inflammation and haemostasis

7.5.1 C-reactive protein and serum amyloid A

C-reactive protein is probably the best-studied circulating inflammatory marker. It is produced by hepatocytes during the acute phase response, under stimulation from inflammatory cytokines that include IL1, IL-6 and TNF-α[229]. It is a highly sensitive but non-specific marker of events that trigger the acute phase response. CRP levels can rise by several orders of magnitude above baseline in response to major infections, inflammation, tissue damage or other stresses[229]. These large rises in CRP should be distinguished from modest elevations above baseline (to levels previously considered negative or normal) that have been found to be associated with significant changes in vascular risk[236]. It is unclear whether CRP plays a direct role in the pathogenesis of inflammation or whether it is simply a marker for inflammatory disorders. This controversy is highlighted by studies in cardiovascular disease: on one hand, the finding that inherited genetic variations in CRP are not linked to coronary heart disease suggests that CRP may not play a causal role[237]; on the other, a number of trials of statins for both primary and secondary prevention of cardiovascular events, in which statin use was associated with major reductions in CRP as well as in serum lipids, suggest that reducing CRP may reduce risk of cardiovascular events[238–240].

Serum amyloid A (SAA) is another acute phase protein synthesised primarily in the liver in response to stimulation from pro-inflammatory cytokines[241]. Levels are raised after infection, injury and other tissue damage. SAA also correlates

with cardiovascular disease[242]. As with CRP, it is unclear whether SAA contributes causally to the development of atherosclerosis and myocardial infarction or simply acts as a marker of underlying disease processes[241]. It is expressed locally at the site of atherosclerotic lesions by macrophages, smooth muscle cells and endothelial cells[243]. Known effects of SAA include the promotion of thrombosis by inducing tissue factor[244], stimulation of production of pro-inflammatory cytokines[244], induction of matrix metalloproteinases[245] and promotion of chemotaxis for monocytes and neutrophils[246]. Recent evidence suggests that SAA also modulates the anti-inflammatory function of high density lipoprotein[247]. Though it is expressed in adipose tissue, its role there is less well understood.

7.5.2 Interleukin-6 and tumour necrosis factor-α

IL-6 is expressed and released from various cells including monocytes, macrophages and endothelial cells upon induction by vasoactive peptides, reactive oxygen species and other cytokines[248]. It is an important proximal mediator of the acute phase response[234] and has a major role in stimulating hepatic secretion of other acute phase proteins including CRP[227] and SAA[248]. It interacts with other inflammatory cytokines including TNF-α, IL-1 and IL-8 to drive inflammation[248]. While many previous studies have focussed on downstream inflammatory mediators such as CRP as these are more stable, long-term average levels of IL-6 have also recently been found to be independently associated with coronary heart disease risk[234]. In addition, IL-6 levels are increased in AMI patients compared to healthy controls[249]. As before, whether IL-6 plays a causative role in coronary heart disease and, by extension, whether it could be used as a potential therapeutic target remains to be seen[234]. Statin trials suggest a reduction in IL-6 levels[250] but the pleiotropic effects of statins may make it difficult to disentangle the significance of this result.

TNF-α is released rapidly in a preformed soluble form from cells such as macrophages, lymphoid cells and endothelial cells in response to inflammatory stimuli[251]. Stimuli include infections and acute tissue injuries such as myocardial ischaemia or infarction. Levels of TNF-α are increased in peripheral blood

samples from AMI patients compared to healthy controls[249] and there may be a low-grade increase in concentration with age[252]. TNF-α works in concert with other pro-inflammatory cytokines, acting on macrophages, monocytes, endothelial cells and vascular smooth muscle cells to promote changes such as increased vascular permeability, leukocyte attachment and transmigration, increased cholesterol uptake, formation of foam cells and increased blood clotting[251]. In high concentrations, TNF-α may contribute to ischaemic tissue damage and myocardial necrosis through triggering fibrosis, apoptosis and hypertrophy[251]. Although antibodies directed against TNF-α have been posited as a potential treatment for cardiovascular conditions, large randomised controlled trials as yet show no evidence of benefit for treatment of cardiac failure[253,254], though it is hypothesised that this may be due to immune system redundancy.

7.5.3 P-selectin and Von Willebrand factor

P-selectin is a trans-membrane inflammatory cell adhesion molecule present in the alpha granules of platelets and Weibel-Palade bodies of endothelial cells[255]. Cell adhesion molecules belong to one of three main families – selectins, immunoglobulins and integrins. In general selectins mediate rolling and binding of flowing blood cells on vascular endothelium, while immunoglobulins such as ICAM-1,2,3 and integrins such as β1-integrin and β 2-integrin, facilitate adhesion and extravasation of blood cells[256]. After activation and rapid translocation to the cell surface, P-selectin initiates interactions of leukocytes and platelets with endothelium[257,258] through its ligand at sites of inflammation and tissue injury. It contributes to the pro-thrombotic environment by up-regulating tissue factor and other pro-coagulant molecules[259]. P-selectin is also involved in platelet-platelet interactions that stabilise platelet aggregates[260]. Both platelet-platelet and platelet-leukocyte interactions are important for the development of arterial thrombosis. P-selectin is a marker of platelet activation in vivo and its levels are raised in various vascular conditions such as acute coronary syndromes, atrial fibrillation, stroke and peripheral vascular disease[255]. Knockout mouse studies suggest it is also critical for the progression of atherosclerosis[261].

Von Willebrand factor is a large plasma protein synthesised in endothelial cells and megakaryocytes and stored in Weibel-Palade bodies and platelet alpha granules[262]. Levels increase markedly during acute-phase responses to systemic or local inflammation[263] and VWF is a key player in the primary haemostatic response to vascular injury[262]. Specific actions include promoting rapid platelet adhesion to exposed collagen within the subendothelium through glycoprotein receptors Ib and IIb/IIIa and to other platelets[206]. VWF is also involved in carriage of factor VIII[206]. It is highly correlated with other markers of both haemostasis and inflammation[227]. A previous meta-analysis has found a moderate relationship between VWF and coronary heart disease, which holds even after adjusting for other markers of haemostasis and inflammation[264].

7.6 Exploratory study

7.6.1 Aims & objectives

Aim: To perform an exploratory study examining markers of inflammation and platelet activation in AMI patients.

Objectives

1. To describe laboratory markers of inflammation and platelet activation in a cohort of AMI patients hospitalised during the 2009 influenza pandemic
2. To investigate whether AMI patients with evidence of recent influenza had higher levels of inflammatory and haemostatic markers than patients without evidence of recent influenza infection.

7.7 Methods

7.7.1 Overview of study design

This single centre study was an extension of the study described in chapter 6. The group of patients hospitalised with AMI during the 2009 influenza pandemic (who were 'cases' in the previous chapter) had an extra serum and plasma sample taken for analysis of levels of inflammatory and haemostatic markers. In this new

study, the exposure was recent influenza infection (defined both clinically and by serological testing) and outcome variables were the various inflammatory and haemostatic markers.

7.7.2 Setting and participants

As before, the study was conducted at the Royal Free Hospital, London, UK between 21/09/2009 and 28/02/2010. Participants were recruited from the acute cardiology ward and coronary care unit. They were eligible for inclusion if aged ≥40 years and admitted with an AMI (defined as before as a rise in troponin T with ischaemic symptoms and/or typical ECG changes, or by angiographic evidence of acute coronary artery thrombosis during primary percutaneous coronary intervention). Exclusion criteria were patients transferred from other hospitals, those unable to provide informed consent, those unable to speak sufficient English or those judged by the treating clinician to be too unwell to take part. The recruitment and consent processes are described in section 6.4.3.

7.7.3 Data sources and management

Demographic and clinical data on the admission, cardiovascular risk factors, symptoms of respiratory illness and influenza vaccination status were obtained through a questionnaire and verified where possible through medical records – see table 6.1 for further details.

For this study in addition to the serum sample taken for determination of the concentration of influenza A IgA antibodies by ELISA, another 5ml serum sample and a 5ml plasma EDTA sample were collected. These were taken to the virology laboratory for storage at -80°C prior to batch testing for a range of inflammatory and haemostatic markers using commercially available assays described below.

7.7.3i Influenza A IgA antibodies

Assays for influenza antibodies were performed by staff in the Virology Department at the Royal Free Hospital. Antibodies were quantified using a

commercially obtained ELISA kit (Biosupply UK, cat no. RE56501), described in detail in section 6.4.5.

7.7.3ii C-reactive protein and serum amyloid A

CRP was measured by staff in the Biochemistry Department at the Royal Free Hospital using a high sensitivity automated microparticle enhanced latex turbidimetric immunoassay (COBAS MIRA; Roche Diagnostics GmbH) and SAA was measured by latex nephelometry (BNII autoanalyser; Dade Behring, Marburg, Germany)[265]. Standardisation of CRP and SAA assays was based on the respective WHO International Reference Standards[266,267]. Results were reported in mg/L.

7.7.3iii Quantitative analysis of cytokines and haemostatic markers

Concentrations of IL-6, TNF-α and soluble P-selectin were measured by staff in the Virology Department at the Royal Free Hospital using immunoassay kits (R&D Systems, cat no's D6050, DTA00C & BBE6) and VWF collagen binding activity (CBA) was quantified using a Technozym VWF: CBA ELISA (Technoclone Ltd, cat no. 5450301). All tests were based on the quantitative sandwich enzyme immunoassay technique. Here, a specific monoclonal antibody against the molecule of interest was pre-coated onto a microplate. Standards and samples were pipetted into wells allowing the molecule to bind to the antibody. Washes were done to remove unbound substrate and then an enzyme-linked polyclonal antibody specific to the desired molecule was added. After further washes a substrate solution was added to wells and colour developed in proportion to the amount of bound molecule present. The optical density of colour was measured within a standard time frame and the equivalent concentration of the molecule read from a standard curve. Minimum detectable doses of IL-6, TNF-α and soluble P-selectin were <0.7pg/ml, 1.6pg/ml and <0.5ng/ml respectively. The collagen binding activity of VWF was measured rather than VWF concentration as this corresponds better to the physiological function of VWF in vivo.

7.7.4 Data management and statistical methods

Categories for demographic and clinical factors were generated as described in section 6.4.8. 'Influenza' was classified clinically as presence or absence of recent

respiratory illness and presence or absence of recent ILI. Influenza antibody concentrations were initially explored as a continuous outcome variable before being grouped by standard thresholds ('positive' >12U/ml, 'equivocal' = 8-12 U/ml and 'negative' <8 U/ml), with equivocal results dropped to leave a binary variable for analysis.

Inflammatory and haemostatic marker outcomes were initially explored as continuous variables with a natural log transformation done to enhance normality of the data distribution. Histograms, ranges and the geometric mean concentration of each marker were described across all study participants and then stratified by presence or absence of recent influenza. p values were presented from t tests of the difference in means of the log of the inflammatory or haemostatic marker concentrations in those with and without evidence of recent influenza. For SAA, the only marker for which log transformation did not produce a standard normal distribution, non-parametric Wilcoxon rank sum tests were also used to test the difference in means.

Univariable linear regression analysis was used to correlate the natural log of each inflammatory or haemostatic marker against each influenza variable as well as against potential confounding factors such as age-group, gender, troponin level (as a marker of the extent of cardiac damage) and type of infarction (STEMI or NSTEMI). As in chapter 6, binary influenza variables were regressed against potential confounding factors using univariable logistic regression analysis. Any factors with Wald test values of $p<0.2$ for their association with both outcome (inflammatory or haemostatic marker) and exposure (influenza) were considered for inclusion in multivariable linear regression models with the main outcome and exposure. Factors were examined in a backwards stepwise procedure using likelihood ratio tests to test the effect of removing each factor sequentially from the model. P values were considered significant at 0.05. As this was a small study it was recognised that there was unlikely to be sufficient power to detect interactions.

7.8 Results

7.8.1 Characteristics of AMI patients

70 patients with AMI were enrolled in the original case control study, of whom 58 had a usable serum or plasma sample for measurement of markers of inflammation and haemostasis. Characteristics of AMI patients with and without laboratory data are shown in table 7.3.

Characteristic of participant	Laboratory data not missing, n (%)	Laboratory data missing, n (%)	P value
Age-group			
40-49	7 (12.1)	1 (8.3)	0.98
50-59	15 (25.9)	4 (33.3)	
60-69	16 (27.6)	3 (250)	
70-79	14 (24.1)	3 (25.0)	
80+	6 (10.3)	1 (8.3)	
Gender			
Male	47 (81.0)	10 (83.3)	0.85
Female	11 (19.0)	2 (16.7)	
Smoker (questionnaire data)			
No never	19 (32.8)	3 (25.0)	0.62
Yes current	23 (39.7)	4 (33.3)	
Yes ex	16 (27.6)	4 (41.7)	
Diabetes			
No	46 (79.3)	10 (83.3)	0.75
Yes	12 (20.7)	2 (16.7)	
Hypertension			
No	24 (41.4)	9 (75.0)	0.03
Yes	34 (58.6)	3 (25.0)	
Hypercholesterolaemia			
No	30 (51.7)	6 (50.0)	0.91
Yes	28 (48.3)	6 (50.0)	
Personal history of AMI			
No	46 (79.3)	10 (83.3)	0.75
Yes	12 (20.7)	2 (16.7)	
Family history of AMI			
No	22 (37.9)	5 (41.7)	0.81
Yes	36 (62.1)	7 (58.3)	
Type of AMI			
STEMI	41 (70.7)	7 (58.3)	0.36
NSTEMI	14 (24.1)	3 (25.0)	
Unclear	3 (5.2)	2 (16.7)	
Influenza vaccination status			
Vaccinated	32 (55.2)	8 (66.7)	0.46
Unvaccinated	26 (44.8)	4 (33.3)	
Respiratory illness			
No	46 (79.3)	7 (58.3)	0.12
Yes	12 (20.7)	5 (41.7)	

Table 7.3 Characteristics of participants with and without missing laboratory data, n=70

7.8.2 Description of inflammatory and haemostatic markers

All samples contained detectable amounts of inflammatory and haemostatic markers. Ranges and geometric mean concentrations of markers for the 58 AMI patients with laboratory data are given in table 7.4. Results are not presented for Von Willebrand factor collagen binding activity: although levels were detectable in all samples, the assay was insufficiently sensitive to distinguish concentrations

above 2 U/ml (and for 43/58 samples the concentration was given as >2U/ml). The histograms below (figures 7.5-7.9) show distributions of outcome variables after log transformation, roughly corresponding to the Normal distribution.

Figure 7.5 Histogram of log of hsCRP concentration (mg/L)

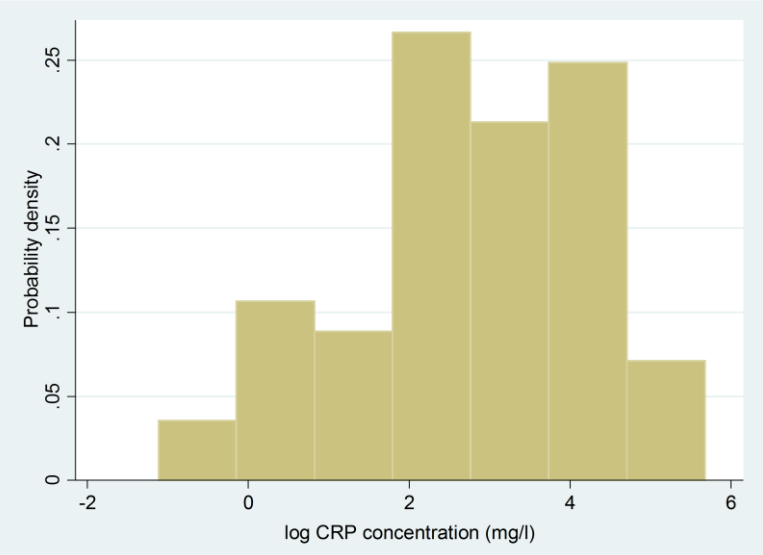


Figure 7.6 Histogram of log of SAA concentration (mg/L)

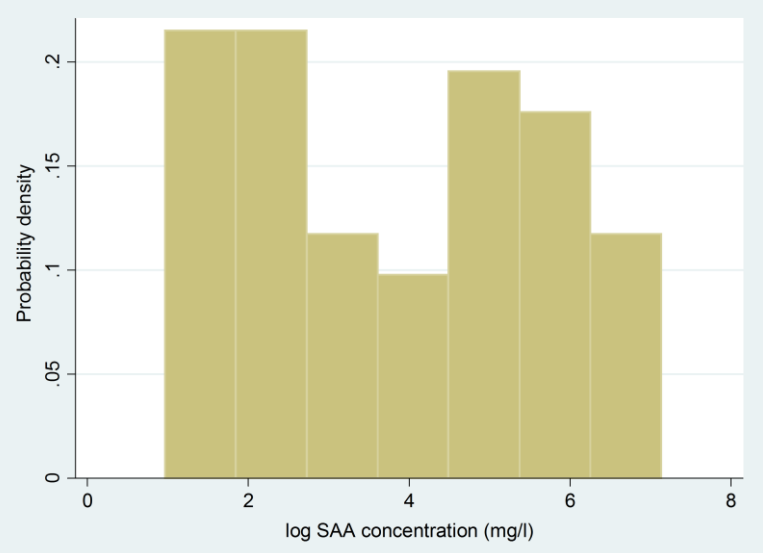


Figure 7.7 Histogram of log of IL-6 concentration (pg/L)

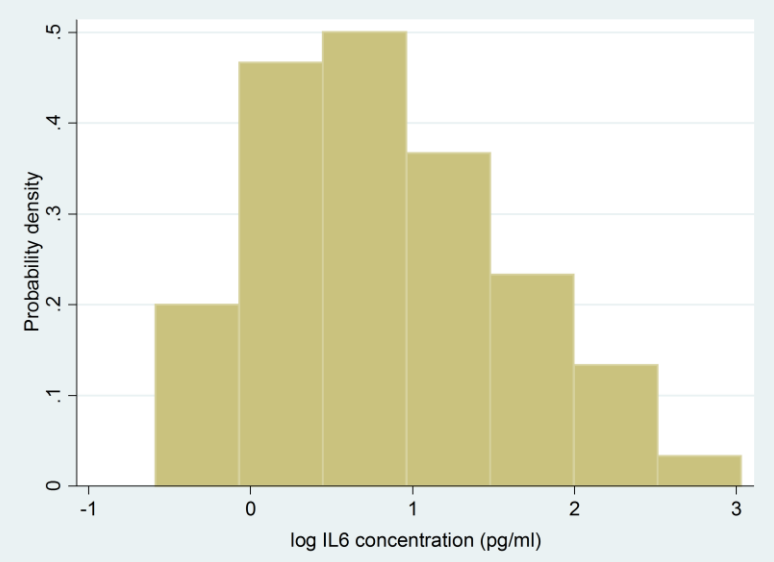


Figure 7.8 Histogram of log of TNF-α concentration (pg/L)

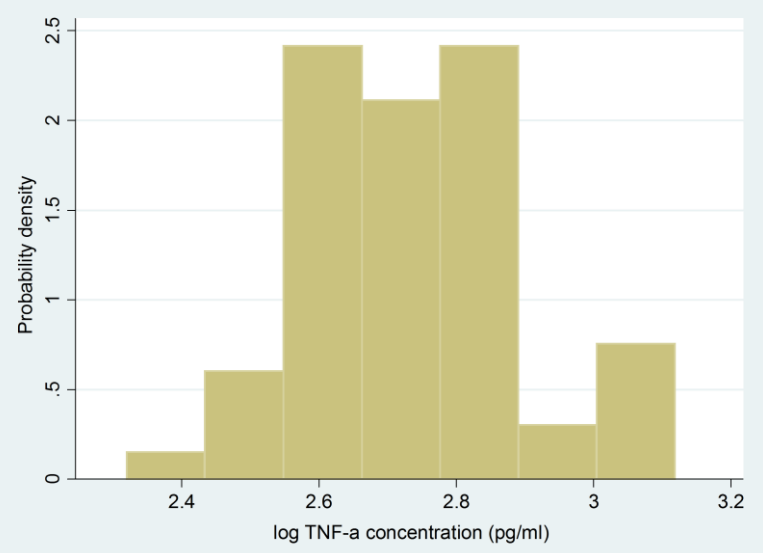
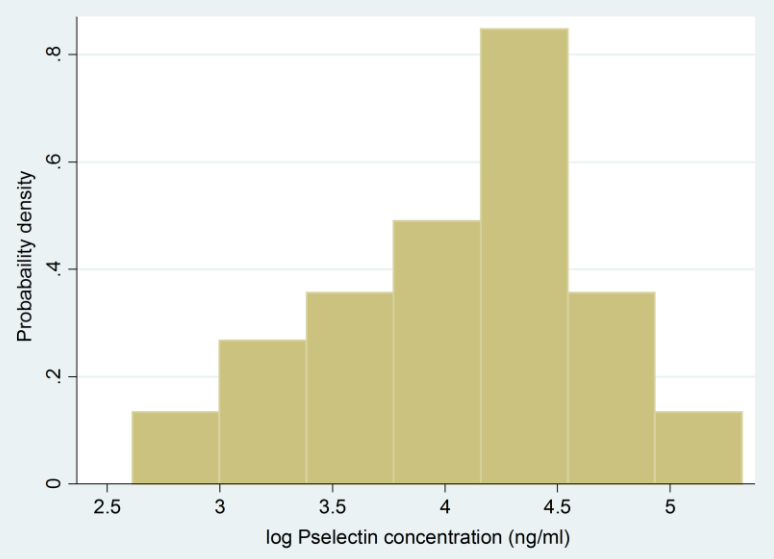


Figure 7.9 Histogram of log of soluble P-selectin concentration (ng/ml)



7.8.3 Univariable associations between inflammatory or haemostatic markers and influenza

Associations between geometric mean concentrations of serum inflammatory and haemostatic markers in AMI patients with and without evidence of recent influenza are shown in table 7.5. For most markers, concentrations were not significantly associated with influenza exposures. An exception was TNF-α, where there was weak evidence that patients with recent respiratory illness (p=0.035) or ILI (p=0.08) had higher TNF-α concentrations. There was similarly weak evidence that patients with influenza IgA antibodies in serum had lower SAA concentrations, both by t test (p=0.07) and Wilcoxon rank sum test (p=0.08).

Result	hsCRP (mg/L)	SAA (mg/L)	IL-6 (pg/ml)*	TNF-a* (pg/ml)	P-selectin* (ng/ml)
Range (min-max)	0.3-293	2.6-1260	0.6-20.7	10.2-22.6	13.6-204.2
Geometric mean (95% CI)	15.6 (10.6 -23.0)	45.3 (27.8-73.9)	2.3 (1.9-2.8)	15.3 (14.6-16.0)	58.5 (50.4-68.0)

Table 7.4 Ranges and geometric mean concentrations of inflammatory and haemostatic markers

Influenza exposure variable	Inflammatory or haemostatic marker outcome variable									
	hsCRP mean (95% CI)	P value	SAA mean (95% CI)	P value	IL-6 mean (95% CI)	P value	TNF-α mean (95% CI)	P value	P-selectin mean (95% CI)	P value
Recent respiratory illness										
Yes	14.7 (7.18-30.2)	0.87	42.1 (13.5-131.2)	0.88	2.2 (1.3-3.6)	0.76	16.7 (15.0-18.8)	0.035	67.0 (44.6-100.5)	0.36
No	15.9 (10.0-17.0)		46.2 (26.3-80.8)		2.3 (1.8-3.0)		14.9 (14.3-15.7)		56.5 (48.0-66.5)	
Influenza like illness										
Yes	15.0 (6.4-35.4)	0.94	33.9 (8.0-143.6)	0.67	1.8 (1.0-3.3)	0.41	17.0 (14.2-20.3)	0.08	71.1 (34.3-147.7)	0.34
No	15.7 (10.2-24.2)		47.1 (27.6-80.5)		2.4 (1.9-3.0)		15.0 (14.4-15.8)		57.0 (49.0-66.3)	
Presence of influenza IgA antibodies										
Yes	10.7 (5.6-20.3)	0.14	26.7 (12.0-59.7)	0.07	2.1 (1.5-2.9)	0.36	15.2 (14.1-16.3)	0.56	57.0 (45.1-71.9)	0.70
No	19.6 (11.4-33.7)		68.4 (35.0-134.0)		2.6 (1.9-3.5)		15.6 (14.7-16.6)		60.6 (48.5-75.6)	

Table 7.5 Geometric mean concentrations of inflammatory and haemostatic markers stratified by recent ARI, ILI and influenza IgA antibodies. P values shown from t test for difference in mean log concentrations

7.8.4 Associations between outcomes and potential confounders

Table 7.6 shows p values for the strength of association between logs of inflammatory or haemostatic marker concentrations (outcomes) and potential confounding factors. Age group was associated with all five outcome variables. Gender and diabetes were associated with three, month of admission, smoking status and peak troponin were associated with two, while personal history of myocardial infarction, family history of stroke and influenza vaccination status were associated with one each. Although personal history of stroke appeared to be associated with four outcome variables, this was based on very small numbers.

Potential confounder	**Inflammatory or haemostatic marker outcome variable**				
	hsCRP	SAA	IL-6	TNF-α	P-selectin
Agegroup	**0.14**	**0.11**	**0.05**	**0.03**	**0.16**
Gender	**0.004**	**0.03**	**0.03**	0.73	0.69
Month of admission	0.88	0.74	0.78	**0.03**	**0.12**
Ethnicity	0.98	0.83	0.48	0.55	0.72
Smoker (questionnaire)	**0.06**	**0.03**	0.22	0.88	0.41
Smoker (medical records)	0.24	**0.10**	0.76	0.75	0.46
Diabetes	**0.16**	**0.08**	0.33	**0.008**	0.65
Hypertension	0.97	1.00	0.38	0.34	0.78
Hypercholesterolaemia	0.32	0.40	0.26	0.91	0.67
Personal history of AMI	**0.19**	0.32	0.30	0.37	0.87
Personal history of stroke	**0.19**	**0.15**	**0.05**	**0.02**	0.28
Family history of AMI	0.27	**0.16**	0.42	0.88	0.50
Family history of stroke	0.74	0.97	0.65	0.59	**0.09**
BMI category	0.77	0.32	0.99	0.52	0.23
Influenza vaccination status	0.60	0.41	0.97	0.69	**0.017**
Peak troponin	**0.13**	**0.06**	0.67	0.98	0.47
Type of AMI	0.96	0.84	0.59	0.23	0.91

Table 7.6 p values for associations between inflammatory or haemostatic marker outcomes and potential confounders.

Factors with a p value <0.2 are shown in **bold** and were considered for inclusion in multivariable models.

7.8.5 Associations between influenza exposures and potential confounders

Table 7.7 (below) shows p values for the strength of associations between four influenza exposures and potential confounding factors. Age group, smoking status, influenza vaccination status and peak troponin were associated with two influenza exposures each while gender, month of admission, personal history of AMI, personal history of stroke, family history of AMI and body mass index were each associated with one influenza exposure.

Potential confounder	**Influenza exposure**			
	Respiratory illness	Influenza-like illness	Presence of antibodies	Antibody concentration
Agegroup	**0.09**	**0.12**	0.81	0.50
Gender	0.31	0.74	0.46	**0.16**
Month of admission	0.24	0.37	0.80	**0.13**
Ethnicity	0.57	0.91	0.30	0.36
Smoker (questionnaire)	0.97	**0.19**	0.27	**0.06**
Smoker (medical records)	0.73	0.41	**0.13**	**0.07**
Diabetes	0.68	0.59	0.72	0.88
Hypertension	0.53	0.47	0.85	0.57
Hypercholesterolaemia	0.89	0.62	0.79	0.73
Personal history of AMI	0.68	0.59	**0.06**	0.44
Personal history of stroke	-	-	-	**0.005***
Family history of AMI	**0.10**	-	0.21	0.70
Family history of stroke	0.83	0.43	-	0.28
BMI category	0.82	0.53	**0.13**	0.92
Influenza vaccination status	0.69	0.91	**0.009**	**0.018**
Peak troponin	**0.04**	**0.15**	0.30	0.90
Type of AMI	0.72	0.77	0.77	0.20

Table 7.7 p values for associations between influenza exposures and potential confounders

Factors with a p value <0.2 are shown in **bold** and were considered for inclusion in multivariable models.

7.8.6 Regression models

7.8.6.i CRP

Linear regression models exploring the association between log hs-CRP concentration and four influenza exposures are shown in table 7.8. In univariable analysis there was no association between recent respiratory illness, recent ILI or concentration of influenza IgA antibodies and log hsCRP concentration, and only a trend towards a negative association between influenza IgA seropositivity and log hs-CRP concentration (p=0.14). In multivariable models, there was a significant negative association between influenza IgA seropositivity and log hs-CRP concentration after adjusting for age group, gender and smoking status (p=0.012).

7.8.6.ii SAA

Similar models exploring the association between log SAA concentration and four influenza exposures are shown in table 7.9. As before, in univariable analysis there was no association between recent respiratory illness, recent ILI or concentration of influenza IgA antibodies and log SAA concentration, and only a trend towards a negative association between influenza IgA seropositivity and log SAA concentration (p=0.07). In multivariable models, however, there was a significant negative association between influenza IgA seropositivity and log SAA concentration after adjusting for age group, gender and smoking status (p=0.004).

Influenza exposure	Coefficient (95% CIs) from univariable analysis	P value	Coefficient (95% CIs) from multivariable analysis	P value
a) Respiratory illness	-0.08 (-1.04-0.89)	0.87	-0.27 (-1.18-0.64)	0.56
b) Influenza-like illness	-0.04 (-1.24-1.15)	0.94	-0.09 (-1.17-0.99)	0.87
c) Influenza A IgA seropositivity	-0.61 (-1.42-0.21)	0.14	-0.90 (-1.59—0.21)	0.012
d) Influenza antibody concentration	0.01 (-0.02-0.03)	0.45	0.00 (-0.03-0.02)	0.71

Table 7.8 Uni- and multivariable models for the association between log hs-CRP concentration and influenza exposures. Multivariable models adjusted for gender (a), gender and smoking status (b & d) and age group, gender and smoking status (c).

Influenza exposure	Coefficient (95% CIs) from univariable analysis	P value	Coefficient (95% CIs) from multivariable analysis	P value
a) Respiratory illness	-0.09 (-1.31-1.13)	0.88	As before	
b) Influenza-like illness	-0.33 (-1.84-1.19)	0.67	-0.20 (-1.58-1.17)	0.77
c) Influenza A IgA seropositivity	-0.94 (-1.96-0.07)	0.07	-1.31 (-2.18—0.45)	0.004
d) Influenza antibody concentration	0.01 (-0.03-0.04)	0.67	-0.01 (-0.04-0.02)	0.48

Table 7.9 Uni- and multivariable models for the association between log SAA concentration and influenza exposures. Multivariable models adjusted for age group, gender and smoking status (b & c) and gender and smoking status (d).

7.8.6.iii IL-6

Table 7.10 shows uni- and multivariable linear regression models of the association between the log of IL-6 concentration and four influenza exposures. There were no significant associations.

7.8.6.iv TNF-α

Similar uni- and multivariable models are shown in table 7.11 for the outcome log TNF-α concentration and the same influenza exposures. Significantly increased TNF-α concentrations were seen for participants with evidence of recent respiratory illness (p=0.04 after adjusting for age group and month) and for those with recent ILI (p=0.04 after adjusting for age group and month). There was no association between influenza IgA antibodies (either seropositivity or concentration) and log TNF-α concentration.

7.8.6.v P-selectin

Univariable linear regression models for associations between the final outcome, log P-selectin concentration, and influenza exposures are shown in table 7.12. There were no significant associations in univariable analysis and no potential confounders were significantly associated with both outcome and exposure so multivariable models were not constructed.

Influenza exposure	Coefficient (95% CIs) from univariable analysis	P value	Coefficient (95% CIs) from multivariable analysis	P value
a) Respiratory illness	-0.08 (-0.61-0.45)	0.76	-0.10 (-0.61-0.41)	0.70
b) Influenza-like illness	-0.27 (-0.93-0.38)	0.41	-0.23 (-0.86-0.39)	0.46
c) Influenza A IgA seropositivity	-0.21 (-0.67-0.25)	0.36	-0.22 (-0.65-0.21)	0.31
d) Influenza antibody concentration	0.01 (0.00-0.02)	0.18	0.01 (-0.01-0.02)	0.33

Table 7.10 Uni- and multivariable models for the association between log IL-6 concentration and influenza exposures. Multivariable models adjusted for age group and gender (a, b & c), and gender (d).

Influenza exposure	Coefficient (95% CIs) from univariable analysis	P value	Coefficient (95% CIs) from multivariable analysis	P value
a) Respiratory illness	0.11 (0.01-0.22)	0.035	0.11 (0.01-0.21)	0.04
b) Influenza-like illness	0.12 (-0.01-0.25)	0.08	0.12 (0.01-0.25)	0.04
c) Influenza A IgA seropositivity	-0.03 (-0.12-0.07)	0.56	-0.01 (-0.09-0.08)	0.91
d) Influenza antibody concentration	0.001 (-0.002-0.003)	0.71	0.000 (-0.003-0.002)	0.97

Table 7.11 Uni- and multivariable models for the association between log TNF-a concentration and influenza exposures. Multivariable models adjusted for age group and month (a, b & c) and age group, month and stroke history (d).

Influenza exposure	Coefficient (95% CIs) from univariable analysis	P value	Coefficient (95% CIs) from multivariable analysis	P value
a) Respiratory illness	0.17 (-0.20-0.54)	0.36	As before	
b) Influenza-like illness	0.22 (-0.24-0.68)	0.34	As before	
c) Influenza A IgA seropositivity	-0.06 (-0.38-0.25)	0.70	As before	
d) Influenza antibody concentration	-0.01 (-0.01-0.00)	0.26	As before	

Table 7.12 Uni- and multivariable models for the association between log P-selectin concentration and influenza exposures. No multivariable models were generated.

7.9 Discussion

7.9.1 Summary of main findings

This exploratory study of the effect of recent influenza on concentrations of various prominent inflammatory and haemostatic markers in AMI patients showed several interesting results: first, participants with recent respiratory illness or ILI had higher levels of TNF-α than those without influenza; second, there was a negative association between seropositivity for influenza A IgA and hs-CRP levels; third, there was a similar negative association between influenza A IgA seropositivity and SAA levels, all after adjustment for relevant confounders. Nevertheless findings should be interpreted with caution given the small study size.

7.9.2 Study strengths

Laboratory outcome measures were robust: freshly frozen samples were prepared in an ideal way; assays generated plausible results within specified reference ranges and all samples yielded a result. The commercial immunoassays for IL-6, TNF-α and P-selectin are well-validated and have been used extensively in previous studies[268–270]. Measuring recent influenza was done (as in chapter 6) using both clinical and laboratory definitions to maximise available data. The feasibility of this approach to collecting and testing blood samples for inflammatory and haemostatic markers has been demonstrated.

7.9.3. Study weaknesses – roles of chance, bias and confounding

This was an exploratory study nested within the case control study described in chapter 6, so the outcomes used here – levels of inflammatory and haemostatic markers – were not primary outcomes. Therefore the study was underpowered to detect differences in these secondary outcomes between those with and without evidence of recent influenza. Although use of several measures of influenza exposure was potentially a strength, each measure had potentially for inaccuracy – eg there was a potential risk of recall bias for recent respiratory

illness (though it seems unlikely that this would differ by inflammatory or haemostatic marker status) and there was uncertainty as to whether presence of influenza A IgA antibodies represented recent infection or recent vaccination. While data were collected on a range of potential confounders, the presence of chronic conditions was not fully explored in questionnaires. For example, chronic conditions tend to be associated with more severe cases and complications of influenza and chronic inflammatory conditions or their treatments could plausibly affect levels of inflammatory and haemostatic markers.

7.9.4 Interpretation of results

Assuming that significant results were not due to factors described above then they represent something of a paradox. On one hand, the raised levels of TNF-α seen in those with recent respiratory or influenza-like illness are to be expected, given the direct triggering effect of respiratory illnesses on acute inflammatory pathways that stimulate production of cytokines such as TNF-α. On the other, it is surprising that this association is not reflected in other inflammatory and haemostatic markers (although given that most reported respiratory symptoms occurred 8-14 days previously and that some inflammatory markers such as IL-6 have a very short half-life in vivo, this may not be so surprising). In fact there is an inverse association between presence of influenza A IgA antibodies and levels of two inflammatory markers hs-CRP and SAA. This is also counter-intuitive as it would be expected that recent influenza would produce higher levels of inflammation. Even influenza vaccine acts as a small inflammatory stimulus so antibody production as the result of vaccination does not explain this result.

Few previous studies have examined inflammatory and haemostatic markers in AMI patients in the context of influenza infection. One case control study of AMI patients and outpatient controls in a Chinese population demonstrated higher levels of a panel of pro-inflammatory cytokines in AMI patients compared to control subjects[249]. AMI patients were also more likely to have serum IgG antibodies to influenza A and B than controls. No direct investigation was done between influenza and cytokine levels in AMI patients, however, and as both

influenza and AMI are independently associated with inflammation it is difficult to draw valid conclusions from these findings.

7.9.5 Future directions

Further basic science studies are needed to establish molecular mechanisms between influenza and AMI. In practice this type of study has been limited by the lack of suitable animal models: ApoE and LDL receptor deficient mice are useful models of chronic atherosclerosis but plaque rupture rarely occurs[209]. While clinical mechanistic studies might be technically more feasible, interpretation of results remains a challenge. To aid understanding, it would be helpful to compare with results of serial measurements of inflammatory and haemostatic markers in patients infected with confirmed influenza. This type of study may be difficult: biased results would be obtained from patients hospitalised with severe influenza; identifying patients with influenza in the community is difficult and time-consuming. Volunteer challenge studies, in which patients are deliberately infected with viruses such as influenza under controlled conditions, are carried out regularly in the UK and might assist in elucidating mechanisms. Randomised controlled trials of the effect of anti-inflammatory and antiviral medications in patients with influenza on AMI would provide further indirect evidence for some of the mechanistic pathways described above.

SUMMARY

- Influenza could theoretically trigger AMI either through direct infection or inflammation on coronary vessels or by acting through indirect systemic inflammatory, haemostatic or haemodynamic mechanisms
- An exploratory study was carried out to measure CRP, SAA, TNF-α, IL-6, P-selectin and Von Willebrand factor collagen binding activity in serum and plasma samples provided by 58 AMI patients from the case control study described in the previous chapter
- Though AMI patients with recent respiratory or influenza-like illness had higher levels of TNF-α than those without recent ARI, results should be interpreted with caution given the study size.

8. Discussion, conclusions, recommendations

8.1 Description of chapter contents

In this chapter I summarise the background to the hypothesis that influenza and acute respiratory infections can trigger acute cardiovascular events and review the rationale for work in this thesis. I draw together findings from this body of research and previous studies, consider key methodological strengths and limitations and assess the level of evidence generated. I explore implications for clinical and public health policy and practice and highlight future research needs.

8.2 Summary of research undertaken

8.2.1 Background and rationale

AMI is a leading cause of death worldwide. Management of AMI remains a key challenge, especially as AMI risk is incompletely explained by traditional vascular risk factors[4]. There is now widespread recognition of the role of inflammation in the pathogenesis of atherosclerosis and AMI[7]. The contribution of inflammatory triggers such as acute respiratory infections, however, remains poorly understood. Influenza may lead to cardiovascular complications, either through direct infection or inflammation of myocardial tissues or through indirect systemic inflammatory and haemodynamic effects[109]. Some cardiac complications of influenza such as myocarditis are well-recognised, but the relationship with AMI is less clear. Improved understanding of this relationship will help to inform seasonal and pandemic influenza planning as well as strategies for AMI prevention.

My work in this thesis aimed to investigate the relationship between acute respiratory infections – particularly influenza – and AMI, building on previous studies to address some earlier methodological limitations such as inadequate control for environmental confounders in population level studies and lack of specificity for influenza in individual-level studies.

8.2.2 Summary of methods and results

Findings from a systematic literature review (chapter 2) tended to support the hypothesis that acute respiratory infections – and influenza in particular – could trigger AMI, with more limited evidence for an association with cardiovascular death. An ecological time series study (chapter 3) showed associations between seasonal influenza circulation and AMI after adjusting for temporal and environmental confounders in England & Wales and Hong Kong. Self-controlled case series studies using electronic health records from the GPRD (chapter 4) and linked records in GPRD, MINAP and HES (chapter 5) found a substantially increased AMI risk after consultation for acute respiratory infection.
In the linked database study AMI risk was greatest for infections judged most likely to be due to influenza. An association between recent ILI and AMI was seen in a hospital-based case control study (chapter 6) conducted during the 2009 influenza A H1N1 pandemic. Potential biological mechanisms were investigated through an exploratory study of cytokines and inflammatory markers in AMI patients with and without recent acute respiratory infection (chapter 7).

As supported by publications listed on p5, this work has contributed substantially to the evidence that acute respiratory infections and influenza in particular are important contributors to AMI. Key findings are highlighted below, placed in the context of previous literature and major strengths and limitations of approaches used are discussed.

8.3 Key findings

1. Acute respiratory infections trigger AMI

8.3.1i Supporting evidence

i. In the present self-controlled case series study in GPRD there was a 3.65-fold increased risk of AMI in the first three days after GP consultation for

acute respiratory infection. This tapered over time suggesting that the increased AMI risk was transient.

ii. Repeating the study using linked MINAP data to classify and time cardiac events more accurately revealed a more pronounced risk with a 4.19-fold increase in AMI risk immediately following consultation for acute respiratory infection.

iii. Five of seven previous observational studies (six case control studies[78–83] and one self-controlled case series study[84]) reported statistically significant associations between occurrence of recent respiratory symptoms and AMI, with effect sizes ranging from a 2-fold to nearly 5-fold increase in risk. The two remaining studies did not demonstrate an effect, although one noted an association of fever with AMI[82] and the other suggested that several GP visits for ARI (but not one visit) were associated with AMI risk[79].

iv. Overall these studies provide good evidence to support the hypothesis that acute respiratory infections can act as triggers of AMI.

8.3.1ii Strengths and limitations

v. Some previous studies were limited by lack of power, risks of recall bias (when recent respiratory symptoms were self-reported) and selection bias when using control groups selected for convenience such as people attending or admitted to hospital for conditions other than AMI.

vi. Using the GPRD conferred some major strengths to my work: it is the largest computerised database of longitudinal primary care records worldwide so analyses were well powered; records are from an unselected sample of 8% of the UK population so results should be generalisable to UK residents; data are high quality and diagnoses well-validated which reduced the risk of misclassification error; in particular, respiratory symptoms are recorded by the GP at consultation, rendering recall by participants unnecessary.

vii. A further strength was the addition of linked MINAP and HES data allowing information on accuracy, specificity and timing of an AMI diagnosis to be improved. MINAP provides near complete coverage of hospitalised AMI cases in England & Wales and detailed information is available on timing of symptom onset, investigations and diagnoses. Having accurate data on timing was particularly important for self-controlled case series analysis

using short risk periods in which delayed recording of AMI would bias results towards the null.

viii. Use of the self-controlled case series method (compared to the case control design selected for many earlier studies) had the advantages of eliminating fixed between-person confounding as well as reducing the risk of residual biases due to lack of suitable control subjects. These are both important considerations when using primary care data originally collected for clinical rather than research purposes.

ix. Despite the many advantages to using a large primary care dataset such work is limited to acute respiratory infections for which medical attention is sought. These cases form only the 'tip of the iceberg' of the community burden of disease, and my results may not therefore be generalisable to all respiratory infections. In studies using other observational designs, under-ascertainment of ARI would tend to bias results towards the null through non-differential misclassification of exposure. This is less of an issue though for self-controlled case series in which every patient is required to have both an exposure and outcome event.

2. Seasonal influenza triggers AMI

8.3.2i Supporting evidence

i. In weekly time series analysis, there were strong associations between influenza circulation and AMI hospitalisations and deaths in a subtropical (Hong Kong) and temperate climate (England & Wales) after controlling for temporal and environmental factors

ii. At least 20 previous ecological studies dating from 1932 until 2012 have noted an association between circulating influenza and the broader outcome cardiovascular death (which includes AMI) – see chapter 2 – although effect sizes were not easily compared across studies due to widely varying time periods (and therefore different influenza strains), populations, case definitions and statistical methods used.

iii. At individual level one previous case control study found a significant association between the presence of IgG antibodies to influenza in an unvaccinated population and AMI[91], which would tend to support our

finding of a particular effect of influenza. Three other small early case control studies using antibody tests for influenza in paired sera, however, found no association with AMI[87–89].

iv. Four small case control studies compared the odds of recent ILI in AMI patients with controls[86–89]. Results were somewhat mixed with two studies reporting significant effects and two reporting slight positive but not significant associations.

v. Overall there is consistent ecological evidence for an association between influenza circulation, ILI and cardiovascular mortality but at individual level the evidence for a specific effect of influenza is less clear.

8.3.2ii Strengths and limitations

vi. Few previous ecological studies adjusted appropriately (or at all) for potential environmental confounders. The risk of ecological bias, where findings at population level fail to reflect individual level associations, was inherent to these studies. Individual level studies were generally small and those using an ILI definition alone lacked specificity for influenza.

vii. The England & Wales/ Hong Kong study extends previous work by taking a more robust approach to controlling for environmental variables, in particular adjusting for weekly mean temperature using flexible natural cubic spline functions, rather than either omitting temperature control or comparing effects in cold versus warm periods, as was done in some earlier studies.

viii. In addition, using data from two geographically distinct settings characterised by differing populations, climates and patterns of influenza circulation reduced the risk that associations may be attributed to residual confounding from environmental factors.

ix. Although the risk of ecological bias could not be overcome in this time series study, triangulation with individual-level results presented in the rest of the thesis helped to ensure that results were robust.

x. Laboratory PCR data on influenza were used in this study (as primary exposure in Hong Kong and in sensitivity analysis in England & Wales – data not shown) to enhance specificity compared to some previous work based on either antibody detection or clinical definitions alone.

xi. One puzzling aspect to this time series analysis was the inconsistent lag times seen between influenza and AMI outcomes in the different settings. It is unclear whether these reflect differences in speed of health-seeking behaviour or reporting delays in surveillance data, and further studies would be needed to disentangle the effect.

3. A triggering effect on AMI may be greater for influenza than for other respiratory infections

8.3.3i Supporting evidence

i. In my self-controlled case series study using linked databases, infections occurring when influenza was circulating and those coded as ILI were associated with consistently higher incidence ratios for AMI, suggesting that a triggering effect might be stronger for influenza than other infections

ii. As described in key findings 1 and 2, there is clear evidence for a triggering effect of general acute respiratory infections on AMI and some evidence for a particular effect of influenza. Nonetheless, no previous study has directly compared the magnitude and strength of the effect of influenza with that of other acute respiratory infections.

iii. Overall further evidence is needed to confirm whether there is a stronger effect of influenza compared to other respiratory infections, ideally using laboratory confirmation of diagnosis.

8.3.3ii Strengths and limitations

iv. Strengths of my analysis included using three complementary methods (levels of circulating influenza, medical codes used to classify infections and influenza vaccination status) to judge which infections were most likely to be due to influenza. The most powerful method – harnessing the high positive predictive value of acute respiratory infections, in particular ILI, for influenza during peak times of influenza circulation – demonstrated the most convincing differential effect.

v. A limitation to using GPRD data to identify acute respiratory infections was that I relied upon GPs' diagnoses based on clinical symptoms and did not have microbiological confirmation of influenza. Although inferences could

be made about which infections were more or less likely to be due to influenza, some misclassification would be inevitable. This is likely to have been non-differential, reducing the size of the apparent effect of influenza on AMI compared to other respiratory infections.

4. AMI risk is highest in the first three days after acute respiratory infection and persists for around a month

8.3.4i Supporting evidence

i. In both of the present self-controlled case series a gradient of AMI risk was evident after ARI. Risks were highest in the first three days and tapered over time to reach baseline by 28 days.

ii. One previous self-controlled case series study in GPRD found a similar gradation of AMI risk after consultation for systemic acute respiratory infection[84]. In this study there was a 5-fold increase in risk at days 1-3, with some increase above baseline persisting for up to 28 days.

iii. Two other large primary care database studies using case control designs support the finding of a graded risk of acute respiratory infection on AMI, strongest immediately after GP consultation, with the highest risk seen for days 1-5 in one study[80] and days 1-7 in the other[83].

iv. A case crossover study of AMI patients with recent ILI showed that the relative probability of an AMI happening on the first rather than the seventh day after infection onset was around 2.5[85].

v. Literature on biological mechanisms suggests that a triggering effect is likely to be transient, given that acute respiratory infections result in a variety of short-lived local and systemic inflammatory and haemodynamic responses that may contribute to atherosclerotic plaque destabilisation[209].

vi. Although the duration of effects varied, the general finding that increased risks are transient is likely to be robust as it is both biologically plausible and consistent across all studies that used graded risk periods.

8.3.4ii Strengths and limitations

vii. Previous studies have used different designs and risk periods after ARI so effect sizes and durations are not directly comparable between studies.

viii. A strength of my study was the use of self-controlled case series analysis with the same graded risk periods after acute infection as a previous similarly designed study, to allow direct comparisons to be made. Self-controlled case series also allowed easy manipulation of time periods: I was also able to exclude the period from ARI consultation up to 14 days before from baseline time, as an AMI occurring in that time window may affect subsequent likelihood of attending the GP (and thereby artificially inflate AMI incidence in baseline periods).

ix. One potential limitation is that I did not have the onset date of ARI but instead used the date of GP consultation. As this is likely to be some time after the onset of respiratory symptoms the true effect on AMI risk may be even greater than that observed, and may last for longer (although this needs further investigation).

5. A triggering effect on AMI is also seen for pandemic H1N1 influenza

8.3.5i Supporting evidence

i. In my case control study, patients hospitalised with AMI during the second wave of the 2009 influenza A H1N1 pandemic were more likely than acute surgical controls to have experienced recent ILI and other key respiratory illness symptoms, although differences were not statistically significant.

ii. There have been no similar analytical studies conducted in the pandemic. Descriptive studies including individual case reports and case series have reported cardiac complications of H1N1pdm09 such as myocarditis and AMI[178,179]. One modelling study estimated that globally there were 83,300 deaths from cardiovascular causes attributed to influenza during the first 12 months of the 2009 influenza pandemic[121].

iii. It is therefore likely that, as with other influenza strains, H1N1pdm09 may be able to trigger AMI, but further adequately powered evidence from studies conducted in a range of populations is needed.

8.3.5ii Strengths and limitations

iv. Previous descriptive studies have not compared with control groups and case reports/ case series tend to be subject to publication bias, so it is difficult to draw conclusions based on these findings.

v. My study was underpowered to detect an effect, as there was a mismatch between age groups typically affected by AMI and those infected with H1N1pdm09. This would not have been the case for seasonal influenza strains which were circulating when this study was designed.

vi. Although I collected laboratory data, which was potentially a strength, serology results in this study were difficult to interpret, partly due to difficulty distinguishing rises in antibody titres caused by infection from those caused by vaccination as well as lack of validation of the IgA assay used against the pandemic influenza strain. The ideal would have been acute and convalescent sera for IgG but this was not practical within resource constrains.

6. The proportion of AMI deaths due to seasonal influenza ranges from 3-5%, rising to 13% in periods of highest influenza circulation

8.3.6i Supporting evidence

i. In my time series study the proportion of AMI-associated deaths attributed to influenza ranged from 3.9-5.6% in Hong Kong and 3.1-3.4% in England & Wales depending on the model of seasonality used. Figures for AMI hospitalisations were lower in both settings. During the most active periods of influenza virus circulation, up to 13% of AMI deaths and 8% of AMI hospitalisations were attributable to influenza.

ii. One ecological study from Colombia estimated that rates of cardiovascular mortality were 8% higher in times of peak influenza circulation than in other periods[119], which accords with these findings.

iii. Other previous ecological studies have tended to estimate the proportion of excess influenza mortality attributed to cardiovascular disease rather than looking specifically at the proportion of AMI deaths associated with influenza so it is not possible to compare figures.

iv. Overall our estimated proportions were similar across sensitivity analyses using different methods to model seasonality and suggest that a small but significant proportion of AMI burden may be attributed to influenza. Nonetheless further work is needed in different settings to establish a likely normal range for this proportion.

8.3.6ii Strengths and limitations

v. A strength of this estimate was that it provided a more meaningful and specific measure of population impact of influenza on AMI than previous estimates based on proportions of influenza excess mortality due to cardiovascular diseases

vi. Nevertheless only two settings and one 10-year time period were investigated and it is recognised that figures are likely to vary depending on setting, time period and circulating influenza strains.

vii. It was also not possible to determine population attributable risk in my individual-level studies due to under-ascertainment of ARI in primary care data.

7. The relative risk of AMI after acute respiratory infection is highest in the elderly

8.3.7i Supporting evidence

i. In both self-controlled case series analyses AMI risks increased with age and were greatest in people aged ≥80 years.

ii. In my time series analysis, incidence rate ratios for AMI were highest in those aged ≥80 years in England & Wales and in Hong Kong.

iii. While a lower age limit of eg 40 years has been imposed in several previous individual-level studies, none has stratified the effect of acute respiratory infection on AMI by age. In five earlier ecological studies using age-specific data[30,65,71,73,77], the strongest associations between AMI and CVD death were noted in the oldest age-groups.

iv. From studies of biological mechanisms, low-grade inflammation is a hallmark of the ageing process[271]. Therefore if influenza acts through inflammatory mechanisms to trigger AMI it is plausible that effects might

be greater in elderly populations where pro-inflammatory phenotypes are already apparent.

v. Overall a consistent age gradient in the effect of acute respiratory infections and influenza on AMI has been demonstrated across many population-level and two individual-level studies, suggesting that risks are highest in the elderly.

8.3.7ii Strengths and limitations

vi. There is a lack of age-specific data from previous individual-level studies.

vii. A strength of my GPRD-based studies was the use of robust measures of age: age checks were conducted as part of a suite of quality control measures before patient records were deemed acceptable for use in research.

viii. A limitation of my time series study was that I did not have access to age-specific data on influenza or ILI so based stratifications on age at AMI. Although this might have enhanced the risk of ecological bias (if much circulating influenza was present in younger age groups), the consistency in findings between this and other studies reduces the risk that the effect was due to bias.

8. Influenza vaccine protects against AMI

8.3.8i Supporting evidence

i. There was a trend towards a protective effect of influenza vaccination against AMI in my pandemic case control study although this was not statistically significant.

ii. In the updated meta-analysis of three previous small RCTs of influenza vaccination in patients with existing vascular disease[102,105,126] there was a 33% reduction in AMI and a 54% reduction in cardiovascular death in vaccinated subjects, with effects just failing to reach statistical significance. If this level of effect were true, it would signify both that influenza vaccination as secondary prevention compared highly favourably with

other risk reduction strategies and that in people with prior cardiovascular disease influenza was responsible for a high proportion of AMI.

iii. Previous observational vaccine studies have shown mixed results. Of 12 studies using cohort, case control or self-controlled case series designs, six demonstrated a protective effect[92,93,97,122,124,125], one showed a protective effect that just failed to reach significance[96] and five found no effect of influenza vaccination[81,84,94,95,123] against a range of cardiac outcomes.

iv. Overall, based mainly on findings from the meta-analysis of RCT data, it is likely that influenza vaccination is effective as secondary prevention against cardiovascular death and AMI. Further evidence is needed on the cardioprotective effects of influenza vaccination in populations without evidence of existing cardiovascular disease.

8.3.8ii Strengths and limitations

v. Previous randomised controlled trials have been small and conducted only in populations with existing cardiovascular disease. Previous observational studies have been limited by the risk of 'healthy user' bias.

vi. None of my studies aimed to elucidate the protective effect of influenza vaccination against AMI as a primary aim. Although I examined this question in my pandemic case control study, influenza vaccination was a secondary exposure and the study was therefore not powered to detect an effect. In this study there was also a risk of misclassification of exposure as influenza vaccine status was determined by self-report.

8.3.9 Summary of evidence generated

Overall there was good evidence for a transient triggering effect of acute respiratory infections, including influenza, on AMI. This was greatest in the first few days following ARI onset and highest in the elderly. A small but important proportion of AMIs was due to influenza. There was some evidence that influenza vaccination is effective at protecting against cardiovascular death and AMI in people with existing vascular disease. The variety of research methods and influenza definitions used in this thesis allowed results to be triangulated across

different data sources, populations and influenza strains. Results were broadly consistent across studies, enhancing their reliability.

8.4 Implications for clinical and public health policy and practice

8.4.1 General

i. Interventions targeted either at preventing acute respiratory infections and influenza in established risk groups or at reducing cardiovascular complications associated with these infections would mitigate the acute triggering effect on AMI and cardiovascular deaths in vulnerable populations.

ii. General non-pharmaceutical measures, such as practising good hand and respiratory hygiene and social distancing measures for people who are ill, may help to reduce the burden of acute respiratory infections in populations[272]. Such non-specific and untargeted measures are, however, likely to have little impact on ARI-associated AMI.

8.4.2 Influenza vaccination

iii. Influenza vaccination offers more specific protection against AMI triggered by influenza rather than by general acute respiratory infections, although its effectiveness at preventing infections is limited to 60-80% in healthy adults[39] and is lower in the elderly. It may also reduce the incidence of severe complications in individuals who become infected with influenza[40].

iv. Two types of policy are relevant to use of influenza vaccine to prevent influenza-associated AMI. First, seasonal and pandemic influenza plans, both at national and international levels recommend that high risk individuals including people aged over 65 as well as those with chronic cardiac conditions and diabetes receive annual influenza vaccination[40,113]. Second, some national policies relating to secondary prevention of AMI such as the American Heart Association/ American College of Cardiology

Foundation guideline recommend that all patients with cardiovascular disease should have an annual influenza vaccination[114].

v. In the UK, however, this recommendation is not included in National Institute for Health and Clinical Excellence (NICE) clinical guideline on secondary prevention of myocardial infarction[273] (due to be updated in November 2013) nor the Scottish Intercollegiate Guidelines Network (SIGN) guidelines on management of acute coronary syndrome[274] or risk estimation and prevention of cardiovascular disease[275].

vi. Evidence from this thesis suggests that this disparity should be addressed to improve patient management and streamline international standards.

vii. My research findings also highlight the need to encourage influenza vaccination uptake in the elderly and those with existing cardiovascular disease: in England influenza vaccine uptake was 74.0% for people aged 65 years and over in the 2011/12 winter season and only 51.6% in people aged 6 months to 65 years in high risk groups[41].

viii. It remains unclear whether influenza vaccination should be used as primary prevention from AMI in people who are at increased cardiovascular risk but fall outside current priority groups for vaccination. Further trials in 'healthy' populations are needed. These could lead to adoption of a risk-based approach for influenza vaccination similar to that used for other preventive therapies for AMI, such as statins, where eligibility is determined by long-term cardiovascular event risk.

8.4.3 Antiviral drugs

ix. Antiviral drugs such as oseltamavir could in theory reduce the risk of influenza-associated AMI either by preventing influenza or by lessening its severity. Antiviral drugs are not available for other common respiratory infections.

x. Currently NICE and the Department of Health recommend that antiviral medications are available as treatment for patients at risk of complications who present within 48 hours of ILI onset[115]. These include all those eligible to receive influenza vaccination as well as patients outside these groups deemed at high risk of complications[40]. Seasonal influenza plans

do not include the use of antiviral medications as prevention but this may differ in the event of an influenza pandemic.

xi. In England there is no standardised method to monitor antiviral uptake during ILI. Research evidence suggests that community uptake of antivirals is extremely low even in times of heightened awareness and wider prescription such as the 2009 influenza pandemic[276].

xii. The effectiveness of antiviral drugs at reducing AMI and cardiovascular complications associated with influenza and other acute respiratory infections is also not known.

xiii. Additional research is needed before the likely impact of antiviral drugs on influenza-associated AMI can be assessed.

8.4.4 Anti-thrombotic treatments during acute respiratory infections

xiv. Interventions to reduce AMI risk are typically prescribed according to estimates of long-term (eg 10-year) cardiovascular risk based on algorithms that incorporate traditional demographic, behavioural and biological risk factors[48,49]. Pharmacological agents including aspirin, beta-blockers, ACE inhibitors and statins have proven benefits in terms of cardiovascular risk reduction[277] which are likely to apply equally to influenza-associated cardiovascular events.

xv. This thesis provides evidence that short-term cardiovascular risk fluctuates in response to external stimuli such as influenza, with AMI risk greatest in the first 3 days after infection onset and increased risk persisting for around a month.

xvi. There is currently no policy recommending short-term use of anti-thrombotic agents such as aspirin or statins to reduce the occurrence of acute vascular events at such times of heightened risk[278].

xvii. Existing studies examining the effectiveness of prophylactic statin and beta-blocker therapy during the peri-operative period (a time of short-term increased vascular risk) are inconclusive[279,280].

xviii. Further research is needed into effectiveness, cost effectiveness and safety before such an approach could be adopted.

8.4.5 Public and health professional awareness of symptoms

xix. Effective management of AMI requires prompt recognition of cardinal symptoms and a rapid primary and secondary care response.

xx. Studies in this thesis have highlighted particular risk periods for AMI such as during times of influenza circulation and following onset of acute respiratory symptoms. Elderly people have also been identified as a high risk population for AMI associated with acute respiratory infection or influenza.

xxi. This suggests that future public health campaigns aimed at increasing cardiac symptom recognition might be most effective when timed to coincide with periods of highest risk and targeted to populations at highest risk such as the elderly and their carers.

8.5 Future research directions

i. While studies in this thesis have identified the elderly as a risk group for AMI associated with acute respiratory infection and influenza, the magnitude of increased risk in other groups such as people with individual cardiovascular risk factors remains unclear. Adequately powered stratified analyses using large datasets from different populations are needed to identify other high risk groups.

ii. Linking laboratory data (eg from the HPA's 'Lab base') on positive specimens to general practice datasets would allow the relative effects of influenza versus other respiratory infections to be better delineated.

iii. Further vaccine trials are needed to assess the protective effect of influenza vaccination against AMI and cardiovascular death in people without existing cardiovascular disease but with vascular risk factors and in the general population.

iv. Research is required into methods to increase influenza vaccine uptake in established risk groups, perhaps using findings from this thesis as part of an educational intervention.

v. More evidence is needed, ideally from RCTs, on the effectiveness, risks, benefits and safety of using antiviral drugs to reduce AMI and cardiovascular complications during acute respiratory viral infection

vi. Information is also needed on the use of short-term antithrombotic therapies during infections to answer questions such as which patients would benefit and the optimal choice, dose and timing of therapy.

vii. Further basic science studies would help to elucidate underlying biological mechanisms and aid identification of novel therapeutic targets. Use of several experimental influenza strains including H1N1pdm09 would clarify whether triggering actions are similar across influenza strains.

viii. Finally there is already a small body of literature suggesting that influenza and acute respiratory infection may trigger acute ischaemic stroke. Studies of whether this effect extends to other acute vascular events such as ruptured aortic aneurysm or acute limb ischaemia would help to guide prescription of prophylactic measures for high risk patients.

8.6 Conclusions

Work from this thesis has contributed substantially to understanding the relationship between influenza and AMI with important clinical and public health implications. Overall, there was good evidence for a transient triggering effect of acute respiratory infections, including influenza, on AMI especially in elderly populations. The effect may be stronger for influenza than for other respiratory infections although it is unclear whether this relates to specific biological mechanisms associated with influenza or is a more general effect of illness severity. A protective effect of influenza vaccination against adverse cardiac outcomes has been demonstrated in randomised controlled trials. This suggests that, regardless of underlying mechanism, efforts focussed on reducing the population burden of influenza and its complications would benefit cardiovascular health. Questions remain about which populations would derive most benefit from this strategy as well as the optimal type and delivery of interventions.

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10. Appendices

10.1 Technical appendices

10.1.1 Data extraction specification for GPRD patient records (chapter 4)

Data-set specification - protocol no. 09_034

Authors: R Williams, Dr. T Williams GPRD, MHRA, UK
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Description of cohort as defined in the protocol
The baseline population will consist of all acceptable patients registered with GPRD practices.

Patients will:

a) be registered with a GPRD UTS practice during the study period (01/01/1999 to 31/12/2008)
b) have a record of incident myocardial infarction (MI) that occurs both within their registration period and the study period
c) have at least six months between their registration date and the date of the incident MI

Incident MI will be defined by the list of the Read/OXMIS codes provided by the researcher and agreed with the GPRD research team (**Annex I**). If more than 100,000 patients are identified, a sample will be selected, restricted first to those patients registered at MINAP-linked practices and secondly in order of calendar year of MI (backwards sequentially).

Gprd medcode	Description
203615	ECG: myocardial infarction
207058	Acute transmural myocardial infarction of unspecified site
207059	Other acute myocardial infarction
207064	Atrial septal defect/current complication following acute myocardial infarct
207065	Ventricular septal defect/current complication following acute myocardial infarction
207066	Postoperative transmural myocardial infarction other sites
211073	MYOCARDIAL INFARCT WITH HYPERTENSION
211078	MYOCARDIAL THROMBOSIS
211079	SUBENDOCARDIAL INFARCTION
212602	ECG: posterior/inferior infarct
216008	Cardiac rupture following myocardial infarction (MI)
216009	Posterior myocardial infarction NOS
216010	Inferior myocardial infarction NOS
216011	Acute septal infarction

216012 Microinfarction of heart
216018 THROMBOSIS ATRIUM,AURIC APPEND&VENT/CURR COMP FOLL ACUTE MI
225084 Acute subendocardial infarction
225085 Acute atrial infarction
225097 Subsequent myocardial infarction
225098 Haemopericardium/current complication following acute myocardial infarct
234219 Lateral myocardial infarction NOS
234226 Subsequent myocardial infarction of inferior wall
234227 Certain current complication follow acute myocardial infarct
234228 Rupture chordae tendinae/current complication following acute myocardial infarct
234229 Rupture papillary muscle/current complication following acute myocardial infarct
243246 Attack – heart
243247 Coronary thrombosis
243248 MI - acute myocardial infarction
243249 Acute anterolateral infarction
243255 Postoperative myocardial infarction
252390 Acute infer-olateral infarction
252391 Acute infero-posterior infarction
252395 Subsequent myocardial infarction of unspecified site
252468 [X]Subsequent myocardial infarction of unspecified site
258148 ECG: subendocardial infarct
259269 PERCUT TRANSLUMINAL CORONARY THROMBOLYSIS WITH STREPTOKINASE
261622 Other specified anterior myocardial infarction
261623 Acute antero-apical infarction
261624 Other acute myocardial infarction NOS
261696 [X]Subsequent myocardial infarction of other sites
265787 THROMBOSIS CORONARY WITH HYPERTENSION
270887 Silent myocardial infarction
270888 Acute antero-septal infarction
270889 Acute papillary muscle infarction
270894 RUPTUR CARDIAC WALL W'OUT HAEMOPERICARD/CUR COMP FOL AC MI
270896 Postoperative transmural myocardial infarction anterior wall
270897 Postoperative subendocardial myocardial infarction
270983 [X]Acute transmural myocardial infarction of unspecified site
276474 ECG: antero-septal infarct.
276475 ECG: lateral infarction
279940 Thrombosis - coronary
279944 Subsequent myocardial infarction of other sites
279945 Postoperative transmural myocardial infarction inferior wall
279946 Postoperative transmural myocardial infarction unspecified site
286731 PERCUT TRANSLUM CORONARY THROMBOLYTIC THERAPY- STREPTOKINASE
289040 Heart attack
289041 True posterior myocardial infarction
289148 [X]Other current complications following acute myocardial infarct
294781 ECG: myocardial infarct NOS
298318 Acute myocardial infarction
298319 Anterior myocardial infarction NOS
298320 Acute myocardial infarction NOS
298329 Subsequent myocardial infarction of anterior wall
298330 Postoperative myocardial infarction, unspecified
303765 MYOCARDIAL INFARCT ACUTE WITH HYPERTENSION
303768 MYOCARDIAL INFARCT
303769 MYOCARDIAL INFARCT ACUTE
303770 INFARCT HEART

303771	THROMBOSIS CORONARY
306424	CORONARY INFARCTION
306459	HEART ATTACK
307878	Acute non-Q wave infarction
308452	Diabetes mellitus insulin-glucose infusion acute myocardial infarct
309266	Acute Q-wave infarct
339896	Acute non-ST segment elevation myocardial infarction
341510	Acute ST segment elevation myocardial infarction
344390	Acute postero-lateral myocardial infarction

Annex I: List of Read/OXMIS codes as evidence of incident MI

10.1.2 AMI codes in MINAP (chapter 5)

Code	Label	Definition
1	Myocardial infarction (STEMI)	There will normally be a history consistent with the diagnosis. The diagnosis requires the presence of cardiographic changes of ST elevation consistent with infarction of =>2mm in contiguous chest leads and/or ST elevation of =>1 mm ST elevation in 2 or more standard leads. (New LBBB is included; although ST elevation is usually apparent in the presence of LBBB). There must be enzyme or troponin elevation. Where CK is used the peak value should exceed twice the upper limit of the reference range. Where troponin assay is used the locally accepted cut off value should be used. (See Threatened MI) This group includes all patients with STEMI regardless of whether typical changes were evident on the admission ECG or developed subsequently.
3	Threatened MI	After early reperfusion treatment there may be rapid resolution of existing ST elevation associated with a CK rise less than twice the upper limit of normal or a small troponin release. If only troponin has been measured and is elevated; it is a local decision whether this is recorded as 'Definite infarction' or 'Threatened infarction'.
4	Acute coronary syndrome (troponin positive)/ NSTEMI	ACS troponin positive includes all those patients previously defined as nSTEMI. There must be symptoms consistent with cardiac ischaemia and there will normally be cardiographic changes consistent with this diagnosis. Troponon elevation above locally determined reference level is mandatory.
7	Myocardial infarction (unconfirmed)	This diagnosis must only be applied to patients who die in hospital before biochemical confirmation of infarction can be confirmed.
50	MI (NSTEMI)	Old code (no longer in use)
51	ACS (troponin unspecified)*	Old code (no longer in use)

Annex 2 List of codes in the MINAP discharge diagnosis field considered as AMI
*This code was only considered to denote an AMI when there was separate evidence of a positive cardiac marker result

10.1.3 ARI codes in GPRD (chapter 5)

Codelist 1 Acute respiratory infection codes with a systemic component

Medcode	Description (Read term)
68	Chest infection
312	Acute bronchitis
556	Influenza
572	Pneumonia due to unspecified organism
763	Whooping cough
886	Bronchopneumonia due to unspecified organism
1019	Acute bronchiolitis
1142	Croup
1257	Acute tracheitis
1285	Laryngotracheitis
1382	Acute viral bronchitis unspecified
1576	Pneumonia due to mycoplasma pneumoniae
1849	Lobar (pneumococcal) pneumonia
2157	Flu like illness
2476	Chest cold
2581	Chest infection NOS
3358	Lower resp tract infection
3683	Basal pneumonia due to unspecified organism
3842	Bordetella pertussis
4899	Recurrent chest infection
5202	Viral pneumonia
5324	Atypical pneumonia
5612	Pneumonia due to staphylococcus
5947	Influenza like illness
5978	Acute wheezy bronchitis
6094	Pneumonia or influenza NOS
6124	Acute lower respiratory tract infection
7267	Notification of whooping cough
8318	Lung consolidation
8980	Influenza-like symptoms
9043	Acute pneumococcal bronchitis
9389	Chest infection - viral pneumonia
9639	Lobar pneumonia due to unspecified organism
9953	Tuberculous pneumonia
10086	Pneumonia and influenza
10087	Acute laryngotracheitis
10093	Tracheopharyngitis
11072	Acute purulent bronchitis
11101	Acute tracheobronchitis

11849 Other specified pneumonia or influenza
12061 Pneumonia - Legionella
12423 Pneumonia due to streptococcus
12476 Acute tracheitis without obstruction
13573 Influenza with bronchopneumonia
14791 Influenza with gastrointestinal tract involvement
14976 Viral pneumonia NOS
15761 Bordetella parapertussis
15774 Influenza with laryngitis
15912 Influenza with pneumonia
16120 Acute laryngitis and tracheitis NOS
16287 Chest infection - unspecified bronchopneumonia
16313 Acute tracheitis NOS
16388 Influenza NOS
17025 Chlamydial pneumonia
17185 Acute bronchiolitis with bronchospasm
17359 Chest infection - unspecified bronchitis
17917 Acute bronchiolitis NOS
18451 Acute bronchiolitis due to respiratory syncytial virus
19400 Chest infection - pnemonia due to unspecified organism
19431 Croup
20198 Acute bronchitis NOS
21061 COPD with acute lower respiratory infection
21145 Acute croupous bronchitis
21415 Pharyngotracheitis
21492 Acute haemophilus influenzae bronchitis
22009 Streptococ pneumon/cause/disease classified/oth chapters
22795 Chest infection - other bacterial pneumonia
22835 Bronchiolitis obliterans organising pneumonia
23095 Bacterial pneumonia NOS
23333 Hypostatic pneumonia
23488 Influenza with respiratory manifestations NOS
23546 Pneumonia due to klebsiella pneumoniae
23726 Pneumonia with varicella
24316 Chest infection with infectious disease EC
24356 Hypostatic bronchopneumonia
24471 Acute laryngotracheitis NOS
24800 Acute bacterial bronchitis unspecified
25259 Acute laryngotracheitis without obstruction
25462 Varicella pneumonitis
25694 Pneumonia due to other specified organisms
27519 Pneumonia with pneumocystis carinii
27641 HIV disease resulting in Pneumocystis carinii pneumonia
28634 Other bacterial pneumonia
29166 Chest infection - pneumococcal pneumonia
29273 Acute bronchitis due to parainfluenza virus

29457 Chest infection - influenza with pneumonia
29617 Influenza with pharyngitis
29669 Acute bronchitis and bronchiolitis
30437 Pneumonia with whooping cough
30509 Post operative chest infection
30591 Pneumonia due to pseudomonas
30653 Chest infection - pneumonia organism OS
31024 Mycoplasma pneumoniae [PPLO] cause/dis classifd/oth chaptr
31269 Pneumonia due to respiratory syncytial virus
31363 Influenza with other manifestations NOS
31603 Staphylococcal pleurisy
31689 Bacterial pleurisy with effusion
31886 Acute bronchitis due to mycoplasma pneumoniae
32172 Postmeasles pneumonia
32818 Pneumococcal pleurisy
33478 Viral pneumonia NEC
34251 Pneumonia due to specified organism NOS
34274 Pneumonia with aspergillosis
34300 Postoperative pneumonia
35082 Pneumonia with pertussis
35189 Abscess of lung with pneumonia
35220 Pneumocystosis
35745 Influenza with pneumonia NOS
36675 Pneumonia due to parainfluenza virus
37447 Acute lower respiratory tract infection
37881 Pneumonia due to haemophilus influenzae
40299 Pneumonia - candidal
40498 Pneumonia with infectious diseases EC
41034 Pneumonia with measles
41137 Acute bronchitis or bronchiolitis NOS
41324 Acute laryngitis and tracheitis
41404 Primary pulmonary blastomycosis
42548 Whooping cough NOS
43286 Pneumonia with cytomegalic inclusion disease
43345 Pneumococcal pleurisy with effusion
43362 Acute streptococcal bronchitis
43625 Influenza with other respiratory manifestation
43884 Pneumonia due to bacteria NOS
44842 Bacterial pleurisy with effusion NOS
45072 Cytomegaloviral pneumonitis
45425 Pneumonia due to proteus
46052 Severe acute respiratory syndrome
46157 Influenza with encephalopathy
47295 Pneumonic plague, unspecified
47472 Influenza with other manifestations
47973 Herpes simplex pneumonia

48593 Acute bronchitis due to respiratory syncytial virus
48804 Pneumonia due to haemophilus influenzae
49398 Pneumonia with typhoid fever
49794 Acute neisseria catarrhalis bronchitis
50396 Acute fibrinous bronchitis
50408 Ornithosis with pneumonia
50867 Pneumonia due to other specified bacteria
51398 Pleuropneumonia-like organism (PPLO) infection
52071 Pneumonia with candidiasis
52384 Pneumonia due to other aerobic gram-negative bacteria
52520 [X]Other viral pneumonia
53753 [X]Other pneumonia, organism unspecified
53897 Whooping cough - other specified organism
53947 [X]Pneumonia in viral diseases classified elsewhere
53969 Pneumonia with systemic mycosis NOS
54533 Acute capillary bronchiolitis
54540 Primary pulmonary coccidioidomycosis
54906 Pulmonary cryptococcosis
55646 Acute myocarditis - influenzal
56762 Toxoplasma pneumonitis
57667 Gangrenous pneumonia
58896 Salmonella pneumonia
59951 Pulmonary histoplasmosis
60119 Pneumonia due to Eaton's agent
60299 E.coli pneumonia
60482 Pneumonia with Q-fever
61359 Eaton's agent infection
61623 Pneumonia with actinomycosis
62623 Pneumonia with ornithosis
62632 Influenza with pneumonia, influenza virus identified
63697 Avian influenza virus nucleic acid detection
63763 [X]Other bacterial pneumonia
63858 Pneumonia due to streptococcus, group B
64286 Other whooping cough NOS
64306 Pulmonary actinomycosis
64890 Acute bronchitis due to rhinovirus
65419 Pneumonia due to escherichia coli
65916 Acute bronchitis due to echovirus
66228 Acute bronchiolitis due to other specified organisms
66362 Pneumonia with infectious diseases EC NOS
66397 [X]Other acute lower respiratory infections
67836 Pneumonia due to adenovirus
67901 Pneumonia with nocardiasis
68867 Acute tracheitis with obstruction
69192 Acute exudative bronchiolitis
69352 Streptococcal pleurisy

69782	Pneumonia with other infectious diseases EC
69898	Acute laryngotracheitis with obstruction
70559	Pneumonia with other infectious diseases EC NOS
70710	Primary pneumonic plague
71370	Acute pseudomembranous bronchitis
72182	Pneumonia with salmonellosis
73100	[X]Acute bronchitis due to other specified organisms
73340	Pulmonary nocardiosis
73735	Pneumonia due to pleuropneumonia like organisms
91123	Parainfluenza type 3 nucleic acid detection
91481	Acute pulmonary histoplasmosis capsulati
93010	Staphylococcal pleurisy with effusion
93153	Acute bronchitis due to coxsackievirus
94130	Parainfluenza type 1 nucleic acid detection
94858	Parainfluenza type 2 nucleic acid detection
94930	Avian influenza
96017	Influenza B virus detected
96018	Influenza H3 virus detected
96019	Influenza H1 virus detected
96059	Mycoplasma pneumoniae detected
96286	Human parainfluenza virus detected
97062	Influenza A virus, other or untyped strain detected
97279	[X]Influenza+other manifestations, virus not identified
97605	[X]Influenza+oth respiratory manifestatns,virus not identifd
97936	[X]Influenza+other manifestations,influenza virus identified
98102	Influenza A (H1N1) swine flu
98103	Possible influenza A virus H1N1 subtype
98115	Suspected swine influenza
98125	Suspected influenza A virus subtype H1N1 infection
98129	Influenza due to Influenza A virus subtype H1N1
98143	Influenza A virus H1N1 subtype detected
98156	Influenza H5 virus detected
98257	[X]Flu+oth respiratory manifestations,'flu virus identified
98381	[X]Pneumonia due to other specified infectious organisms
98782	Pneumonia with toxoplasmosis
99214	[X]Acute bronchiolitis due to other specified organisms
100943	[X]Whooping cough, unspecified
101292	Histoplasma duboisii with pneumonia
101507	Histoplasma capsulatum with pneumonia
101775	Acute membranous bronchitis

Codelist 2 As list 1 but minus non influenza organism-specific codes (used to extract episodes where underlying diagnosis could plausibly be influenza)

Medcode	Description (Read term)
68	Chest infection
312	Acute bronchitis
556	Influenza
572	Pneumonia due to unspecified organism
886	Bronchopneumonia due to unspecified organism
1019	Acute bronchiolitis
1142	Croup
1257	Acute tracheitis
1285	Laryngotracheitis
1382	Acute viral bronchitis unspecified
2157	Flu like illness
2476	Chest cold
2581	Chest infection NOS
3358	Lower resp tract infection
3683	Basal pneumonia due to unspecified organism
4899	Recurrent chest infection
5202	Viral pneumonia
5324	Atypical pneumonia
5947	Influenza like illness
5978	Acute wheezy bronchitis
6094	Pneumonia or influenza NOS
6124	Acute lower respiratory tract infection
8318	Lung consolidation
8980	Influenza-like symptoms
9389	Chest infection - viral pneumonia
9639	Lobar pneumonia due to unspecified organism
10086	Pneumonia and influenza
10087	Acute laryngotracheitis
10093	Tracheopharyngitis
11072	Acute purulent bronchitis
11101	Acute tracheobronchitis
11849	Other specified pneumonia or influenza
12476	Acute tracheitis without obstruction
13573	Influenza with bronchopneumonia
14791	Influenza with gastrointestinal tract involvement
14976	Viral pneumonia NOS
15774	Influenza with laryngitis
15912	Influenza with pneumonia
16120	Acute laryngitis and tracheitis NOS
16287	Chest infection - unspecified bronchopneumonia

16313 Acute tracheitis NOS
16388 Influenza NOS
17185 Acute bronchiolitis with bronchospasm
17359 Chest infection - unspecified bronchitis
17917 Acute bronchiolitis NOS
19400 Chest infection - pnemonia due to unspecified organism
19431 Croup
20198 Acute bronchitis NOS
21061 Chronic obstruct pulmonary dis with acute lower resp infectn
21145 Acute croupous bronchitis
21415 Pharyngotracheitis
22835 Bronchiolitis obliterans organising pneumonia
23333 Hypostatic pneumonia
23488 Influenza with respiratory manifestations NOS
24316 Chest infection with infectious disease EC
24356 Hypostatic bronchopneumonia
24471 Acute laryngotracheitis NOS
25259 Acute laryngotracheitis without obstruction
29457 Chest infection - influenza with pneumonia
29617 Influenza with pharyngitis
29669 Acute bronchitis and bronchiolitis
30509 Post operative chest infection
31363 Influenza with other manifestations NOS
33478 Viral pneumonia NEC
34300 Postoperative pneumonia
35189 Abscess of lung with pneumonia
35745 Influenza with pneumonia NOS
37447 Acute lower respiratory tract infection
40498 Pneumonia with infectious diseases EC
41137 Acute bronchitis or bronchiolitis NOS
41324 Acute laryngitis and tracheitis
43625 Influenza with other respiratory manifestation
46157 Influenza with encephalopathy
47472 Influenza with other manifestations
50396 Acute fibrinous bronchitis
52520 [X]Other viral pneumonia
53753 [X]Other pneumonia, organism unspecified
53947 [X]Pneumonia in viral diseases classified elsewhere
54533 Acute capillary bronchiolitis
55646 Acute myocarditis - influenzal
57667 Gangrenous pneumonia
62632 Influenza with pneumonia, influenza virus identified
63697 Avian influenza virus nucleic acid detection
66362 Pneumonia with infectious diseases EC NOS
66397 [X]Other acute lower respiratory infections
68867 Acute tracheitis with obstruction

69192	Acute exudative bronchiolitis
69782	Pneumonia with other infectious diseases EC
69898	Acute laryngotracheitis with obstruction
70559	Pneumonia with other infectious diseases EC NOS
71370	Acute pseudomembranous bronchitis
94930	Avian influenza
96017	Influenza B virus detected
96018	Influenza H3 virus detected
96019	Influenza H1 virus detected
97062	Influenza A virus, other or untyped strain detected
97279	[X]Influenza+other manifestations, virus not identified
97605	[X]Influenza+oth respiratory manifestatns,virus not identifd
97936	[X]Influenza+other manifestations,influenza virus identified
98102	Influenza A (H1N1) swine flu
98103	Possible influenza A virus H1N1 subtype
98115	Suspected swine influenza
98125	Suspected influenza A virus subtype H1N1 infection
98129	Influenza due to Influenza A virus subtype H1N1
98143	Influenza A virus H1N1 subtype detected
98156	Influenza H5 virus detected
98257	[X]Flu+oth respiratory manifestations,'flu virus identified
101775	Acute membranous bronchitis

Codelist 3 Influenza-like illness codes

Medcode	Description (Read term)
556	Influenza
2157	Flu like illness
5947	Influenza like illness
6094	Pneumonia or influenza NOS
8980	Influenza-like symptoms
11849	Other specified pneumonia or influenza
13573	Influenza with bronchopneumonia
14791	Influenza with gastrointestinal tract involvement
15774	Influenza with laryngitis
15912	Influenza with pneumonia
16388	Influenza NOS
23488	Influenza with respiratory manifestations NOS
29457	Chest infection - influenza with pneumonia
29617	Influenza with pharyngitis
31363	Influenza with other manifestations NOS
35745	Influenza with pneumonia NOS
43625	Influenza with other respiratory manifestation
46157	Influenza with encephalopathy
47472	Influenza with other manifestations
55646	Acute myocarditis - influenzal

62632	Influenza with pneumonia, influenza virus identified
63697	Avian influenza virus nucleic acid detection
94930	Avian influenza
96017	Influenza B virus detected
96018	Influenza H3 virus detected
96019	Influenza H1 virus detected
97062	Influenza A virus, other or untyped strain detected
97279	[X]Influenza+other manifestations, virus not identified
97605	[X]Influenza+oth respiratory manifestatns,virus not identifd
97936	[X]Influenza+other manifestations,influenza virus identified
98102	Influenza A (H1N1) swine flu
98103	Possible influenza A virus H1N1 subtype
98115	Suspected swine influenza
98125	Suspected influenza A virus subtype H1N1 infection
98129	Influenza due to Influenza A virus subtype H1N1
98143	Influenza A virus H1N1 subtype detected
98156	Influenza H5 virus detected
98257	[X]Flu+oth respiratory manifestations,'flu virus identified

Codelist 4 As list 1 but minus influenza-like illness codes (for comparison with list 3)

Medcode	Description (Read term)
68	Chest infection
312	Acute bronchitis
572	Pneumonia due to unspecified organism
763	Whooping cough
886	Bronchopneumonia due to unspecified organism
1019	Acute bronchiolitis
1142	Croup
1257	Acute tracheitis
1285	Laryngotracheitis
1382	Acute viral bronchitis unspecified
1576	Pneumonia due to mycoplasma pneumoniae
1849	Lobar (pneumococcal) pneumonia
2476	Chest cold
2581	Chest infection NOS
3358	Lower resp tract infection
3683	Basal pneumonia due to unspecified organism
3842	Bordetella pertussis
4899	Recurrent chest infection
5202	Viral pneumonia
5324	Atypical pneumonia
5612	Pneumonia due to staphylococcus

5978 Acute wheezy bronchitis
6124 Acute lower respiratory tract infection
7267 Notification of whooping cough
8318 Lung consolidation
9043 Acute pneumococcal bronchitis
9389 Chest infection - viral pneumonia
9639 Lobar pneumonia due to unspecified organism
9953 Tuberculous pneumonia
10086 Pneumonia and influenza
10087 Acute laryngotracheitis
10093 Tracheopharyngitis
11072 Acute purulent bronchitis
11101 Acute tracheobronchitis
12061 Pneumonia - Legionella
12423 Pneumonia due to streptococcus
12476 Acute tracheitis without obstruction
14976 Viral pneumonia NOS
15761 Bordetella parapertussis
16120 Acute laryngitis and tracheitis NOS
16287 Chest infection - unspecified bronchopneumonia
16313 Acute tracheitis NOS
17025 Chlamydial pneumonia
17185 Acute bronchiolitis with bronchospasm
17359 Chest infection - unspecified bronchitis
17917 Acute bronchiolitis NOS
18451 Acute bronchiolitis due to respiratory syncytial virus
19400 Chest infection - pnemonia due to unspecified organism
19431 Croup
20198 Acute bronchitis NOS
21061 Chronic obstruct pulmonary dis with acute lower resp infectn
21145 Acute croupous bronchitis
21415 Pharyngotracheitis
21492 Acute haemophilus influenzae bronchitis
22009 Streptococ pneumon/cause/disease classified/oth chapters
22795 Chest infection - other bacterial pneumonia
22835 Bronchiolitis obliterans organising pneumonia
23095 Bacterial pneumonia NOS
23333 Hypostatic pneumonia
23546 Pneumonia due to klebsiella pneumoniae
23726 Pneumonia with varicella
24316 Chest infection with infectious disease EC
24356 Hypostatic bronchopneumonia
24471 Acute laryngotracheitis NOS
24800 Acute bacterial bronchitis unspecified
25259 Acute laryngotracheitis without obstruction
25462 Varicella pneumonitis

25694 Pneumonia due to other specified organisms
27519 Pneumonia with pneumocystis carinii
27641 HIV disease resulting in Pneumocystis carinii pneumonia
28634 Other bacterial pneumonia
29166 Chest infection - pneumococcal pneumonia
29273 Acute bronchitis due to parainfluenza virus
29669 Acute bronchitis and bronchiolitis
30437 Pneumonia with whooping cough
30509 Post operative chest infection
30591 Pneumonia due to pseudomonas
30653 Chest infection - pneumonia organism OS
31024 Mycoplasma pneumoniae [PPLO] cause/dis classifd/oth chaptr
31269 Pneumonia due to respiratory syncytial virus
31603 Staphylococcal pleurisy
31689 Bacterial pleurisy with effusion
31886 Acute bronchitis due to mycoplasma pneumoniae
32172 Postmeasles pneumonia
32818 Pneumococcal pleurisy
33478 Viral pneumonia NEC
34251 Pneumonia due to specified organism NOS
34274 Pneumonia with aspergillosis
34300 Postoperative pneumonia
35082 Pneumonia with pertussis
35189 Abscess of lung with pneumonia
35220 Pneumocystosis
36675 Pneumonia due to parainfluenza virus
37447 Acute lower respiratory tract infection
37881 Pneumonia due to haemophilus influenzae
40299 Pneumonia - candidal
40498 Pneumonia with infectious diseases EC
41034 Pneumonia with measles
41137 Acute bronchitis or bronchiolitis NOS
41324 Acute laryngitis and tracheitis
41404 Primary pulmonary blastomycosis
42548 Whooping cough NOS
43286 Pneumonia with cytomegalic inclusion disease
43345 Pneumococcal pleurisy with effusion
43362 Acute streptococcal bronchitis
43884 Pneumonia due to bacteria NOS
44842 Bacterial pleurisy with effusion NOS
45072 Cytomegaloviral pneumonitis
45425 Pneumonia due to proteus
46052 Severe acute respiratory syndrome
47295 Pneumonic plague, unspecified
47973 Herpes simplex pneumonia
48593 Acute bronchitis due to respiratory syncytial virus

48804 Pneumonia due to haemophilus influenzae
49398 Pneumonia with typhoid fever
49794 Acute neisseria catarrhalis bronchitis
50396 Acute fibrinous bronchitis
50408 Ornithosis with pneumonia
50867 Pneumonia due to other specified bacteria
51398 Pleuropneumonia-like organism (PPLO) infection
52071 Pneumonia with candidiasis
52384 Pneumonia due to other aerobic gram-negative bacteria
52520 [X]Other viral pneumonia
53753 [X]Other pneumonia, organism unspecified
53897 Whooping cough - other specified organism
53947 [X]Pneumonia in viral diseases classified elsewhere
53969 Pneumonia with systemic mycosis NOS
54533 Acute capillary bronchiolitis
54540 Primary pulmonary coccidioidomycosis
54906 Pulmonary cryptococcosis
56762 Toxoplasma pneumonitis
57667 Gangrenous pneumonia
58896 Salmonella pneumonia
59951 Pulmonary histoplasmosis
60119 Pneumonia due to Eaton's agent
60299 E.coli pneumonia
60482 Pneumonia with Q-fever
61359 Eaton's agent infection
61623 Pneumonia with actinomycosis
62623 Pneumonia with ornithosis
63763 [X]Other bacterial pneumonia
63858 Pneumonia due to streptococcus, group B
64286 Other whooping cough NOS
64306 Pulmonary actinomycosis
64890 Acute bronchitis due to rhinovirus
65419 Pneumonia due to escherichia coli
65916 Acute bronchitis due to echovirus
66228 Acute bronchiolitis due to other specified organisms
66362 Pneumonia with infectious diseases EC NOS
66397 [X]Other acute lower respiratory infections
67836 Pneumonia due to adenovirus
67901 Pneumonia with nocardiasis
68867 Acute tracheitis with obstruction
69192 Acute exudative bronchiolitis
69352 Streptococcal pleurisy
69782 Pneumonia with other infectious diseases EC
69898 Acute laryngotracheitis with obstruction
70559 Pneumonia with other infectious diseases EC NOS
70710 Primary pneumonic plague

71370	Acute pseudomembranous bronchitis
72182	Pneumonia with salmonellosis
73100	[X]Acute bronchitis due to other specified organisms
73340	Pulmonary nocardiosis
73735	Pneumonia due to pleuropneumonia like organisms
91123	Parainfluenza type 3 nucleic acid detection
91481	Acute pulmonary histoplasmosis capsulati
93010	Staphylococcal pleurisy with effusion
93153	Acute bronchitis due to coxsackievirus
94130	Parainfluenza type 1 nucleic acid detection
94858	Parainfluenza type 2 nucleic acid detection
96059	Mycoplasma pneumoniae detected
96286	Human parainfluenza virus detected
98381	[X]Pneumonia due to other specified infectious organisms
98782	Pneumonia with toxoplasmosis
99214	[X]Acute bronchiolitis due to other specified organisms
100943	[X]Whooping cough, unspecified
101292	Histoplasma duboisii with pneumonia
101507	Histoplasma capsulatum with pneumonia
101775	Acute membranous bronchitis

10.1.4 Participant information sheet and consent form (chapter 6)

Example participant information sheet (cases)

Royal Free Hampstead NHS
NHS Trust

Study title: The role of influenza as a trigger for acute myocardial infarction (heart attack)

We would like to invite you to take part in a research study, which is being done by Dr Charlotte Warren-Gash as part of a PhD. Before you decide, you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
We are interested in whether infections such as influenza (flu) can trigger heart attacks. Some research suggests that recent infections are more common in people who have had heart attacks. If true, this would help healthcare professionals to reduce the risk of heart attacks by vaccinating people against infections or treating infections promptly when they do occur.

Why have I been invited to take part?
You have been invited to take part because you have had a heart attack. The research team aims to compare the recent experience of infections in people who have had heart attacks compared to people who have not had a heart attack.

Do I have to take part?
It is up to you to decide. We will describe the study and answer any questions that you might have after reading this information sheet. If you agree to take part we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This would not affect the quality of care you receive.

What will happen to me if I take part?
If you take part, the study will involve the following:

1) A short interview with the researcher, which should take no more than 10 minutes. Questions will be asked about recent cough, sore throat or other symptoms of infection, whether you have had a flu vaccination and some questions about your medical history eg. whether you have diabetes.

2) A nasal swab to check for influenza virus.

3) A blood test to check for influenza antibodies and for signs of inflammation.

4) Giving the researcher permission to look in your medical records to find out more about your heart attack eg. what treatment you have had.

5) For some people who are due to come back to clinic, we will ask whether you would mind having a second blood test then (to do a different test for influenza antibodies).

We will also ask your permission to store your blood sample (whether or not it is positive for influenza) for possible future research into heart attacks or influenza and other respiratory infections.

PTO

What are the possible disadvantages and risks of taking part?
There are few risks to taking part in this study. Taking time to answer questions on recent symptoms of infection might be slightly inconvenient, but the interview should last no more than 10 minutes. Having an extra blood test taken might be slightly uncomfortable. However if possible we will aim to take this blood sample at the same time as your normal blood tests to minimise inconvenience.

What are the possible benefits of taking part?
We cannot promise that the study will help you, but information we get from this study may help to prevent others from having heart attacks in future.

Will my taking part in the study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential. You will be given a unique 'participant identification number'. Answers to your interview questions, blood test results and any information collected from your medical records will be stored under that number. You will not be identifiable when results of the study are published.

What will happen if I don't want to carry on with the study?
You are free to withdraw at any time. If you do withdraw from the study, we will destroy all your identifiable samples, but we will need to use data collected up to your withdrawal.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

What will happen to the results of the research study?
We plan to write to each participant to give them the results of their antibody tests for flu. If the results show that you have had flu, this will not require any medical treatment. We can also provide you with a short summary of findings

from the study. We hope to publish the results of this study in a scientific journal.

Further information and contact details

Chief investigator: Dr Charlotte Warren-Gash, MRC Clinical Research Training Fellow, UCL Centre for Infectious Disease Epidemiology.
E-mail: c.warren-gash@pcps.ucl.ac.uk Tel: 020 7830 2239 x36720

Supervisors: Dr Andrew Hayward, Senior Lecturer in Infectious Disease Epidemiology, UCL and Professor Liam Smeeth, Professor of Epidemiology, London School of Hygiene & Tropical Medicine

Collaborators: Dr Anna Maria Geretti, Consultant Virologist; Professor George Hamilton, Professor of Vascular Surgery and Dr Roby Rakhit, Consultant Cardiologist (all at the Royal Free Hospital).

If you would like a large print or audio version of this information, please ask a member of staff.

Study Number: 7848

CONSENT FORM

Title of Project: The role of influenza as a trigger for acute myocardial infarction (heart attack)

Name of Researcher: Dr Charlotte Warren-Gash/ Dr Andrew Hayward

Please initial box

1. I confirm that I have read and understand the information sheet dated 04-08-09 (version 3) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes may be looked at by the researcher. I give permission for the researcher to have access to my records. ☐

4. I agree to my blood sample being stored for possible future research into heart attacks or influenza and other respiratory infections ☐

5. I agree to take part in the above study. ☐

_____ _____ _____
Name of Participant Date Signature

_____ _____ _____
Name of Person taking consent Date Signature

When completed, 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes