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

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-002604
Article Type:	Research
Date Submitted by the Author:	16-Jan-2013
Complete List of Authors:	Warren-Gash, Charlotte; UCL, Research Department of Infection & Population Health Geretti, Anna Maria; University of Liverpool, Institute of Infection and Global Health Hamilton, George; Royal Free London Foundation Trust, Department of Vascular Surgery Rakhit, Roby; Royal Free London Foundation Trust, Department of Cardiology Smeeth, Liam; London School of Hygiene and Tropical Medicine, Epidemiology and Population Health Hayward, Andrew; University College London, Infection and Population Health
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Infectious diseases, Cardiovascular medicine
Keywords:	Myocardial infarction < CARDIOLOGY, Epidemiology < INFECTIOUS DISEASES, PUBLIC HEALTH

Title Influenza-like illness in acute myocardial infarction patients during the winter wave of the influenza A H1N1 pandemic in London: a case control study

Authors Charlotte Warren-Gash[1], Anna Maria Geretti[2], George Hamilton[3], Roby D. Rakhit[4], Liam Smeeth[5], Andrew C. Hayward[1]

Affiliations

[1]Centre for Infectious Disease Epidemiology, Research Department of Infection & Population Health, UCL, London, UK

[2] Institute of Infection and Global Health, University of Liverpool, UK

[3] Department of Vascular Surgery, Royal Free London Foundation Trust, London, UK

[4] Department of Cardiology, Royal Free London Foundation Trust, London, UK

[5] Department of Non-Communicable Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, UK

Corresponding author

Dr Charlotte Warren-Gash

Research Department of Infection & Population Health

1st Floor, Royal Free Hospital

Rowland Hill Street

London NW3 2PF

Email: c.warren-gash@ucl.ac.uk / charlotte.warren-gash@doctors.org.uk

Tel: 020 7794 0500 x36720 Fax: 020 7794 1224

Word Count Abstract 296, main text 2109 (excluding article summary, tables, figures, legends and refs)

ABSTRACT

Objective: To investigate recent respiratory and influenza-like illnesses in acute myocardial infarction patients compared to patients hospitalised for acute non vascular surgical conditions during the second wave of the 2009 influenza A H1N1 pandemic

Design: Case control study

Setting: Coronary care unit, acute cardiology and acute surgical admissions wards in a major teaching hospital in London, UK

Participants: 134 participants (70 cases and 64 controls) aged ≥40 years hospitalised for acute myocardial infarction and acute surgical conditions between 21/09/2009 and 28/02/2010, frequency-matched for gender, 5-year age-band and admission week

Primary exposure: Influenza-like illness (ILI - defined as feeling feverish with either cough or sore throat) within the last month. **Secondary exposures**: Acute respiratory illness within the last month not meeting ILI criteria; nasopharyngeal and throat swab positive for influenza virus

Results: 29 participants of 134 (21.6%) reported respiratory illness within the last month, of whom 13 (9.7%) had illnesses meeting ILI criteria. The most frequently reported category for timing of respiratory symptom onset was 8-14 days before admission (31.0% of illnesses). Cases were more likely than controls to report ILI – adjusted OR 3.17 (95% confidence interval 0.61-16.47) – as well as other key respiratory symptoms, and were less likely to have received influenza vaccination – adjusted OR 0.46 (95% CI, 0.19-1.12) – although differences were not statistically significant. No swabs were positive for influenza virus.

Conclusions: This study was supportive of the hypothesis that recent ILI was more common in patients hospitalised with acute myocardial infarction than with acute surgical conditions during the second wave of the influenza A H1N1 pandemic, and suggestive of a cardio-protective effect of influenza vaccination. It was, however, underpowered, partly because the age groups typically affected by acute

myocardial infarction had low rates of infection with the pandemic influenza strain compared to seasonal influenza.

ARTICLE SUMMARY

Article focus

- Seasonal influenza can trigger cardiovascular complications but the cardiac effects of the 2009 influenza pandemic are less clear.
- We aimed to investigate recent influenza-like illness in patients hospitalised with acute myocardial infarction and surgical conditions during the 2009 influenza pandemic in London.

Key messages

- 14.3% of patients hospitalised with acute myocardial infarction (cases) reported recent influenza-like illness compared to 4.7% of patients hospitalised for acute surgical conditions (controls)
- Cases were more likely than controls to report a range of recent respiratory symptoms and less likely to have received influenza vaccination though differences were not statistically significant
- The median age of cases with acute myocardial infarction was 63.6 years, whereas the majority of people infected with pandemic influenza strain nationally were young

Strengths and limitations

- The study was underpowered to detect an effect, partly due to low infection rates with the pandemic influenza virus in age-groups typically affected by acute myocardial infarction, but it will inform design of future similar studies

INTRODUCTION

Seasonal influenza can trigger cardiovascular complications and deaths in vulnerable populations, especially the elderly and those with underlying medical conditions[1]. Evidence to support the hypothesis that seasonal influenza may trigger acute myocardial infarction comes from a range of observational studies incorporating the effects of different circulating influenza strains and subtypes[2]. 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 [3,4]. 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[5]. Initial evidence from the first wave in the UK suggested that typical illnesses were mild and affected mainly children and young people[6]. The average age of cases increased over subsequent waves of the pandemic[7] but it is unclear how this affected clinical illness profiles. Vaccination coverage did not reach high levels until the post-pandemic season.

There have been reports of myocarditis, myocardial injury and left ventricular systolic dysfunction in patients with severe H1N1pdm09[8,9]. It has been suggested that H1N1pdm09 was associated with higher rates of extra-pulmonary complications than seasonal influenza[10] 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[11], but the contribution of myocardial infarction deaths to this figure is unknown.

In this study we aimed to investigate whether patients hospitalised for acute myocardial infarction during the winter wave of the influenza A H1N1 pandemic were more likely than surgical patients to have experienced recent influenza-like illness or acute respiratory illness, or to have concurrent PCR positive influenza or evidence of influenza A IgA antibodies in sera.

METHODS

Setting, design and participants

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. Cases were patients aged ≥40 years who had experienced 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 myocardial infarction 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.

Exposures

The main exposure was recent influenza-like illness, defined as a history of feeling feverish with either cough or sore throat within the last month. We 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 criteria for influenza-like illness. Additional exposures were nasopharyngeal and throat swabs testing positive for influenza by real-time polymerase chain reaction (PCR), presence of IgA antibodies to influenza A in serum samples and self-reported influenza vaccination status.

Data sources and measurement

We used a questionnaire to investigate recent respiratory and influenza-like illness as well as to collect data on demographics, medical history and influenza vaccination status. Medical records were reviewed for details of the current admission and to confirm data on potential confounding factors. Combined nasopharyngeal and throat swabs were taken from each participant, placed in viral transport medium and transported to the laboratory for storage at-80˚C. Samples were tested for the presence of influenza virus RNA using a validated in house real-time PCR with a lower limit of detection of 1 RNA copy per reaction, as previously described[12]. A single serum sample was taken for quantification of IgA antibodies to influenza A as a marker of recent exposure. Serum samples were centrifuged, frozen at -80˚C and batch tested using a commercially available enzyme-linked immunosorbent assay (EIA) for influenza A IgA (Biosupply UK, cat no. RE56501). Antibody concentrations were initially explored as a continuous 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.

Statistical methods

All analyses were carried out using Stata (*Stata Statistical Software: Release 12. College Station, TX: StataCorp LP*). Baseline comparability between cases and controls was assessed with X^2 tests. 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. We used multivariable logistic regression analysis to investigate associations between recent influenza-like illness or acute respiratory illness exposures and case/ control status, controlling for age-group, gender, month of admission and influenza vaccination status (all models) and other potential confounding factors. These were examined using a backwards stepwise approach whereby factors independently associated with both exposure and outcome were included in models and likelihood ratio tests used to assess the effect of removing each one sequentially. If p values from likelihood ratio tests were <0.1 then factors were retained in the model.

RESULTS

Characteristics of study participants

134 participants were recruited, comprising 70 cases and 64 controls, for whom acceptance rates were 66% and 67% respectively. Median age was 63.6 years (IQR 53.3-72.6) and 21% of participants were female. Cases were more likely to be of Asian or Asian British ethnicity (p=0.016), to have a previous history of myocardial infarction (p=0.04) or a family history of myocardial infarction (p<0.001) than controls. Of the 70 patients hospitalised for acute myocardial infarction, 48 (68.5%) met criteria for ST-elevation myocardial infarction, 17 (24.3%) had a non ST-elevation myocardial infarction and in 5 (7.1%) cases the subtype of myocardial infarction was unspecified. Control patients were admitted with a range of acute non-vascular surgical presentations that included colorectal, urological and orthopaedic conditions.

Table 1

Timing of participants' admissions in relation to national influenza circulation

A comparison of study participants' dates of admission with national rates of GP consultations for influenza-like illness (ILI) based on RCGP surveillance data is shown in figure 1. The peak week for ILI consultations in England was week 43 (ending 25th October 2009) when 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 samples reached 41.2%. Our recruitment period spanned this period of peak influenza circulation.

Figure 1

Recent respiratory and influenza-like illness

17 cases (24.3%) and 12 controls (18.8%) reported respiratory illness in the month preceding hospital admission. 13 illnesses – reported by 10 cases (14.3%) and 3 controls (4.7%) – met criteria for influenza-like illness (defined as feeling feverish with cough and/or sore throat). The most frequently reported category for the timing of respiratory symptom onset was 8-14 days before admission (31.0% of illnesses). 4-7 days was the most frequently reported category for length of illness (37.9% of illnesses). Symptom profiles of participants reporting recent respiratory illness are shown in figure 2. No swabs tested positive for influenza virus nucleic acid. Serum samples were available on 113 of 134 participants (84.3%). There were no significant differences in characteristics of participants with and without missing serum samples (data not shown). 25 cases (43.1%) and 28 controls (50.9%) tested positive for serum influenza A IgA antibodies. 62% of participants who were seropositive had received influenza vaccination compared to 31% of seronegative participants.

Figure 2

Cases were more likely to have reported influenza-like illness than controls – adjusted OR 3.17 (95% confidence interval 0.61-16.47) – as well as other key respiratory illness symptoms, although differences were not statistically significant. There was also a trend towards a protective effect of influenza vaccination against myocardial infarction – adjusted OR 0.46 (95% CI, 0.19-1.12). Results from the logistic regression analysis are summarised in table 2.

Table 2

DISCUSSION

The study was supportive of the hypothesis that recent respiratory and in particular influenza-like illnesses occurring during the second wave of the influenza A H1N1 pandemic were more common in patients hospitalised with acute myocardial infarction than with acute surgical conditions. Influenza vaccination was also associated with protection against myocardial infarction, although differences were not statistically significant. While we 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 rates of influenza-like illness remained low [5], especially in age groups typically affected by acute myocardial infarction. The study was therefore underpowered to detect an effect, partly due to limited numbers of infections among participants.

Using self-reported recent respiratory and influenza-like illness as exposures introduced the possibility of reporting or recall bias. Nonetheless 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[13]. 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. We chose to test both nasopharyngeal and throat swabs to increase the sensitivity of virus detection. It was perhaps unsurprising, however, that none of the nasopharyngeal and throat swabs was positive for influenza virus given a) the low rates of infection in this age-group[5] and b) that the majority of viral shedding in influenza occurs in the first 2-3 days after symptom onset[14] whereas most reported respiratory symptoms in study participants occurred 8-14 days before admission. Based on our findings it seems unlikely that any delayed cardiac effect of influenza is linked to ongoing or prolonged virus replication or shedding in the respiratory tract. 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[15] but its effect with the pandemic strain H1N1pdm09 is unclear. It has previously been noted that serological studies carried out during the 2009 influenza pandemic were severely hampered by cross reactivity both with vaccine and with seasonal influenza strains[7].

Previous observational studies using large electronic primary care databases have found an association between GP consultation for acute respiratory infection in the last month and risk of acute myocardial infarction[16-18]. Although studies were conducted over different time periods, they encompassed the effect of varying seasonal influenza strains. Our recent self-controlled case series study using linked primary care and cardiac disease registry data from 3,927 patients also included acute respiratory infection consultations occurring during the first wave of H1N1pdm09 circulation[19]. We found an incidence ratio for acute myocardial infarction of 4.19 (95% confidence interval 3.18-5.53) in the first 1-3 days after acute respiratory infection, with the risk falling to baseline after 28 days[19]. Elderly people and those consulting for an infection judged most likely to be due to influenza were at greatest risk. During the 2009 influenza pandemic people with underlying cardiovascular disease were much more likely to be hospitalised[13] and to die[20] from a range of causes attributable to pandemic influenza. Although most deaths from H1N1pdm09 occurred in younger people, this was partly a function of the age distribution of infections: in the UK the case fatality rate in the elderly was much higher than in younger age-groups[5] but overall numbers of deaths were small as few elderly people were infected.

Various biological mechanisms are proposed to underlie a relationship between influenza or acute respiratory infection and myocardial infarction[21]. Acute respiratory infections may result in a host of acute inflammatory and haemostatic effects leading to systemic inflammation, altered plasma viscosity, coagulability and haemodynamic changes[22] as well as promoting local endothelial dysfunction, coronary inflammation and plaque rupture[23]. Immobility associated with bed-rest and dehydration might potentiate these processes.

In conclusion, this study suggests that recent ILI occurring during the 2009 influenza pandemic was more common in AMI patients, so indirectly supports the hypothesis that, as with other influenza strains, H1N1pdm09 could potentially trigger AMI in vulnerable groups. The population impact of H1N1pdm09 on rates of hospitalisations and deaths from myocardial infarction, however, is likely to have been relatively low given the mismatch between the ages of those typically affected by H1N1pdm09 and acute coronary events as well as the relatively mild clinical effects of this influenza strain.

Acknowledgments

We thank all patients who agreed to participate in this study and nurses on the cardiology and surgical wards at the Royal Free Hospital who helped to identify potential participants. We acknowledge Mauli Patel at the Royal Free Virology Laboratory who kindly processed, stored and tested samples.

Contributors

CWG conceived and designed the study, recruited participants, collected and analysed data and wrote the manuscript. AMG, GH, RDR, LS and ACH assisted with study design. AMG provided laboratory resources. CWG, AMG, RDR, LS and ACH interpreted results. AMG, GH, RDR, LS and ACH critically revised the manuscript for intellectual content. LS and ACH provided supervision. All authors approved the final version of the manuscript.

Funding statement

This work was funded by a Medical Research Council Clinical Research Training Fellowship for CWG, grant number G0800689. LS is supported by a Wellcome Trust Senior Research Fellowship in Clinical Science, grant number 098504/Z/12/Z.

Competing interests statement

None declared

Data sharing statement

No additional data are available

References

1. Cox NJ, Subbarao K (1999) Influenza. The Lancet 354: 1277–1282.
2. Warren-Gash C, Smeeth L, Hayward AC (2009) Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. The Lancet Infectious Diseases 9: 601–610.
3. Swine influenza A (H1N1) infection in two children – Southern California, March-April 2009 (2009). MMWR Morb Mortal Wkly Rep 58: 400–402.
4. Outbreak of swine-origin influenza A (H1N1) virus infection – Mexico, March-April 2009 (2009). MMWR Morb Mortal Wkly Rep 58: 467–470.
5. Health Protection Agency (2010) Epidemiological report of pandemic (H1N1) 2009 in the United Kingdom: April 2009 - May 2010.
6. McLean E, Pebody RG, Campbell C, Chamberland M, Hawkins C, et al. (2010) Pandemic (H1N1) 2009 influenza in the UK: clinical and epidemiological findings from the first few hundred (FF100) cases. Epidemiol Infect 138: 1531–1541.
7. Lagacé-Wiens PRS, Rubinstein E, Gumel A (2010) Influenza epidemiology – past, present, and future. Crit Care Med 38: e1–e9.
8. Chacko B, Peter JV, Pichamuthu K, Ramakrishna K, Moorthy M, et al. (2012) Cardiac manifestations in patients with pandemic (H1N1) 2009 virus infection needing intensive care. J Crit Care 27: 106.e1–6.
9. Davoudi AR, Maleki AR, Beykmohammadi AR, Tayebi A (2012) Fulminant myopericarditis in an immunocompetent adult due to pandemic 2009 (H1N1) influenza A virus infection. Scand J Infect Dis 44: 470–472.
10. Lee N, Chan PKS, Lui GCY, Wong BCK, Sin WWY, et al. (2011) Complications and Outcomes of Pandemic 2009 Influenza A (H1N1) Virus Infection in Hospitalized Adults: How Do They Differ From Those in Seasonal Influenza? J Infect Dis 203: 1739–1747.
11. Dawood FS, Iuliano AD, Reed C, Meltzer MI, Shay DK, et al. (2012) Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. The Lancet Infectious Diseases 12: 687–695.

12. Khanom AB, Velvin C, Hawrami K, Schutten M, Patel M, et al. (2011) Performance of a nurse-led paediatric point of care service for respiratory syncytial virus testing in secondary care. J Infect 62: 52–58.
13. Campbell CNJ, Mytton OT, McLean EM, Rutter PD, Pebody RG, et al. (2010) Hospitalization in two waves of pandemic influenza A(H1N1) in England. Epidemiology & Infection 139: 1560–1569.
14. Lau LLH, Cowling BJ, Fang VJ, Chan K-H, Lau EHY, et al. (2010) Viral shedding and clinical illness in naturally acquired influenza virus infections. J Infect Dis 201: 1509–1516.
15. Rothbarth PH, Groen J, Bohnen AM, De Groot R, Osterhaus AD (1999) Influenza virus serology – a comparative study. J Virol Methods 78: 163–169.
16. Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, et al. (2004) Risk of myocardial infarction and stroke after acute infection or vaccination. New England Journal of Medicine 351: 2611–2618.
17. Clayton TC, Thompson M, Meade TW (2008) Recent respiratory infection and risk of cardiovascular disease: case-control study through a general practice database. Eur Heart J 29: 96–103.
18. Meier CR, Jick SS, Derby LE, Vasilakis C, Jick H, et al. (1998) Acute respiratory-tract infections and risk of first-time acute myocardial infarction. The Lancet 351: 1467–1471.
19. Warren-Gash C, Hayward AC, Hemingway H, Denaxas S, Thomas SL, et al. (2012) Influenza infection and risk of acute myocardial infarction in England and Wales: a CALIBER self-controlled case series study. J Infect Dis 206: 1652–1659.
20. Mytton OT, Rutter PD, Mak M, Stanton E, Sachedina N, et al. (2012) Mortality due to pandemic (H1N1) 2009 influenza in England: a comparison of the first and second waves. Epidemiology & Infection 140: 1533–1541.
21. Bazaz R, Marriott HM, Francis SE, Dockrell DH (2013) Mechanistic links between acute respiratory tract infections and acute coronary syndromes. J Infect 66: 1-17.
22. Harskamp RE, Van Ginkel MW (2008) Acute respiratory tract infections: a potential trigger for the acute coronary syndrome. Annals of Medicine 40: 121–128.
23. Vallance P, Collier J, Bhagat K (1997) Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? The Lancet 349: 1391–1392.

Figure legends

Figure 1 Number of study participants admitted by influenza surveillance week compared to weekly ILI rates per 100,000 based on national RCGP surveillance data

Figure 2 Percentage of cases (n=70) and controls (n=64) reporting various symptoms during respiratory illness

Tables

Characteristic	Category	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)	
Admission month	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)	
	30.0-39.9	8 (12.3)	10 (17.0)	
	40.0-max	1 (1.5)	2 (3.4)	
Influenza vaccination status[†]	Vaccinated	30 (42.9)	29 (45.3)	0.78
	Unvaccinated	40 (57.1)	35 (54.7)	

Table 1 Characteristics of study participants (n=134)

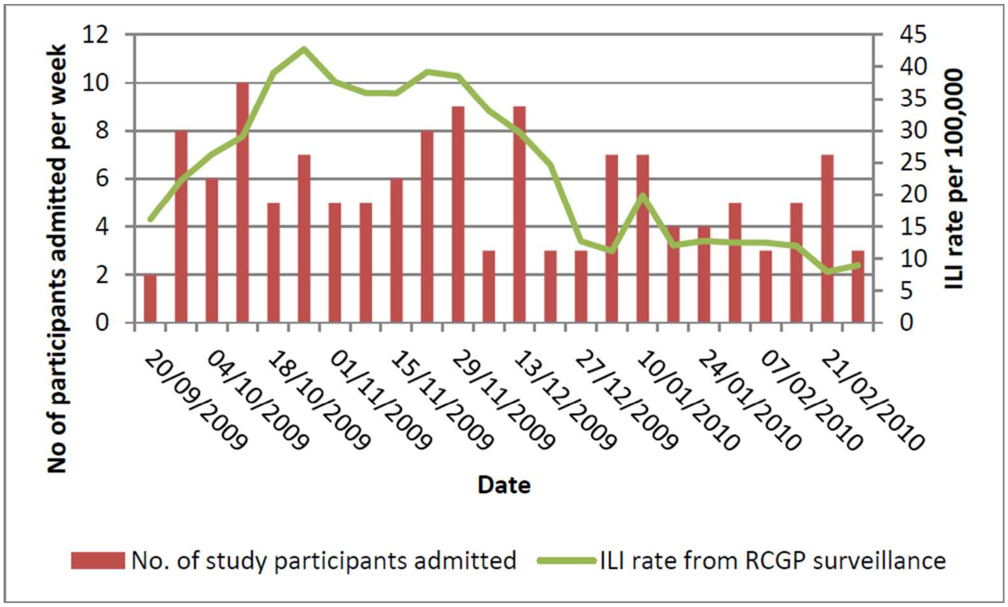
[†]'Vaccinated' refers to receiving influenza vaccination in the current vaccination year (ie since September 2009). All other years were classed as 'unvaccinated' as the H1N1pdm09 strain was not represented in the vaccine

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)

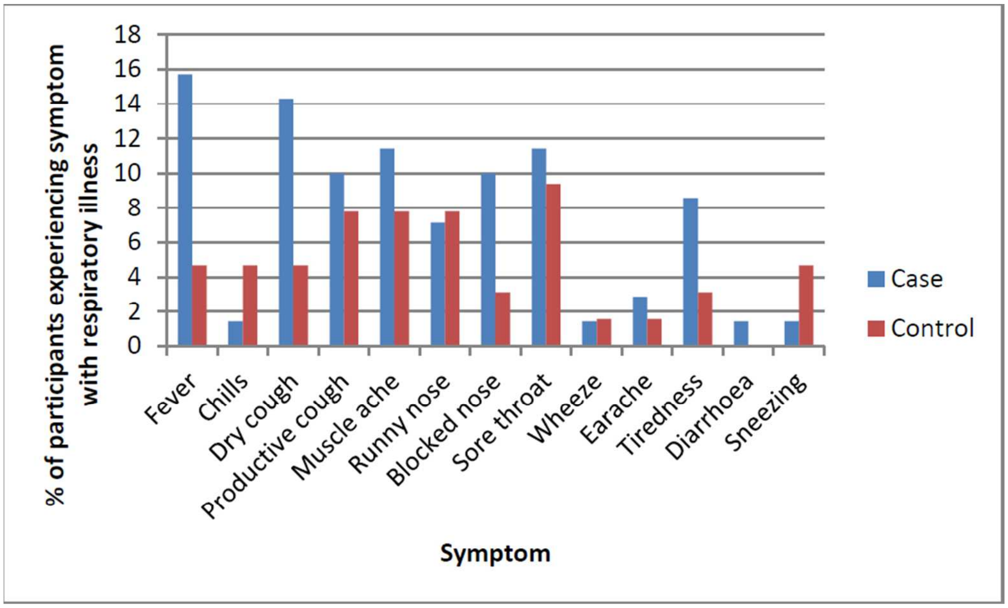
Table 2 Odds ratios for the association between acute myocardial infarction and various respiratory illness exposure variables, unadjusted and adjusted

*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 8 equivocal results were excluded, compared to n=134 (70 cases and 64 controls) for all other exposures.



Number of study participants admitted by influenza surveillance week compared to weekly ILI rates per 100,000 based on national RCGP surveillance data
264x158mm (96 x 96 DPI)



Percentage of cases (n=70) and controls (n=64) reporting various symptoms during respiratory illness
264x158mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Reported on page no.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5
		(*b*) For matched studies, give matching criteria and the number of controls per case	Frequency matching criteria given on p5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	Study size based on numbers of eligible patients hospitalised during the influenza circulation period
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how matching of cases and controls was addressed	N/A

		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	Data not systematically collected but reasons for non-participation include lack of time and feeling unwell during admission
		(c) Consider use of a flow diagram	Not done as no follow up with this study design. Fig 1 shows recruitment over time.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1 and p8
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9-10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-002604.R1
Article Type:	Research
Date Submitted by the Author:	07-Mar-2013
Complete List of Authors:	Warren-Gash, Charlotte; UCL, Research Department of Infection & Population Health Geretti, Anna Maria; University of Liverpool, Institute of Infection and Global Health Hamilton, George; Royal Free London Foundation Trust, Department of Vascular Surgery Rakhit, Roby; Royal Free London Foundation Trust, Department of Cardiology Smeeth, Liam; London School of Hygiene and Tropical Medicine, Epidemiology and Population Health Hayward, Andrew; University College London, Infection and Population Health
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Infectious diseases, Cardiovascular medicine
Keywords:	Myocardial infarction < CARDIOLOGY, Epidemiology < INFECTIOUS DISEASES, PUBLIC HEALTH

SCHOLARONE™ Manuscripts

Title Influenza-like illness in acute myocardial infarction patients during the winter wave of the influenza A H1N1 pandemic in London: a case control study

Authors Charlotte Warren-Gash[1], Anna Maria Geretti[2], George Hamilton[3], Roby D. Rakhit[4], Liam Smeeth[5], Andrew C. Hayward[1]

Affiliations

[1]Centre for Infectious Disease Epidemiology, Research Department of Infection & Population Health, UCL, London, UK

[2] Institute of Infection and Global Health, University of Liverpool, UK

[3] Department of Vascular Surgery, Royal Free London Foundation Trust, London, UK

[4] Department of Cardiology, Royal Free London Foundation Trust, London, UK

[5] Department of Non-Communicable Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, UK

Corresponding author

Dr Charlotte Warren-Gash

Research Department of Infection & Population Health

1st Floor, Royal Free Hospital

Rowland Hill Street

London NW3 2PF

Email: c.warren-gash@ucl.ac.uk / charlotte.warren-gash@doctors.org.uk

Tel: 020 7794 0500 x36720 Fax: 020 7794 1224

Word Count Abstract 296, main text 2389 (excluding article summary, tables, figures, legends and refs)

ABSTRACT

Objective: To investigate recent respiratory and influenza-like illnesses in acute myocardial infarction patients compared to patients hospitalised for acute non vascular surgical conditions during the second wave of the 2009 influenza A H1N1 pandemic

Design: Case control study

Setting: Coronary care unit, acute cardiology and acute surgical admissions wards in a major teaching hospital in London, UK

Participants: 134 participants (70 cases and 64 controls) aged ≥40 years hospitalised for acute myocardial infarction and acute surgical conditions between 21/09/2009 and 28/02/2010, frequency-matched for gender, 5-year age-band and admission week

Primary exposure: Influenza-like illness (ILI - defined as feeling feverish with either cough or sore throat) within the last month. **Secondary exposures**: Acute respiratory illness within the last month not meeting ILI criteria; nasopharyngeal and throat swab positive for influenza virus

Results: 29 participants of 134 (21.6%) reported respiratory illness within the last month, of whom 13 (9.7%) had illnesses meeting ILI criteria. The most frequently reported category for timing of respiratory symptom onset was 8-14 days before admission (31.0% of illnesses). Cases were more likely than controls to report ILI – adjusted OR 3.17 (95% confidence interval 0.61-16.47) – as well as other key respiratory symptoms, and were less likely to have received influenza vaccination – adjusted OR 0.46 (95% CI, 0.19-1.12) – although differences were not statistically significant. No swabs were positive for influenza virus.

Conclusions: Point estimates suggested that recent ILI was more common in patients hospitalised with acute myocardial infarction than with acute surgical conditions during the second wave of the influenza A H1N1 pandemic, and influenza vaccination was associated with cardio-protection, although findings were not statistically significant. The study was underpowered, partly because the age groups typically

affected by acute myocardial infarction had low rates of infection with the pandemic influenza strain compared to seasonal influenza.

ARTICLE SUMMARY

Article focus

- Seasonal influenza can trigger cardiovascular complications but the cardiac effects of the 2009 influenza pandemic are less clear.
- We aimed to investigate recent influenza-like illness in patients hospitalised with acute myocardial infarction and surgical conditions during the 2009 influenza pandemic in London.

Key messages

- 14.3% of patients hospitalised with acute myocardial infarction (cases) reported recent influenza-like illness compared to 4.7% of patients hospitalised for acute surgical conditions (controls)
- Cases were more likely than controls to report a range of recent respiratory symptoms and less likely to have received influenza vaccination though differences were not statistically significant
- The median age of cases with acute myocardial infarction was 63.6 years, whereas the majority of people infected with pandemic influenza strain nationally were young

Strengths and limitations

- The study was underpowered to detect an effect, partly due to low infection rates with the pandemic influenza virus in age-groups typically affected by acute myocardial infarction, but it will inform design of future similar studies

INTRODUCTION

Seasonal influenza can trigger cardiovascular complications and deaths in vulnerable populations, especially the elderly and those with underlying medical conditions[1]. Evidence to support the hypothesis that seasonal influenza may trigger acute myocardial infarction comes from a range of observational studies incorporating the effects of different circulating influenza strains and subtypes[2]. 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 [3,4]. 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[5]. Initial evidence from the first wave in the UK suggested that typical illnesses were mild and affected mainly children and young people[6]. The average age of cases increased over subsequent waves of the pandemic[7] but it is unclear how this affected clinical illness profiles. Vaccination coverage did not reach high levels until the post-pandemic season.

There have been reports of myocarditis, myocardial injury and left ventricular systolic dysfunction, which may be reversible, in patients with severe H1N1pdm09[8,9]. It has been suggested that H1N1pdm09 was associated with higher rates of extra-pulmonary complications than seasonal influenza[10] 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[11], but the contribution of myocardial infarction deaths to this figure is unknown.

In this study we aimed to investigate whether patients hospitalised for acute myocardial infarction during the winter wave of the influenza A H1N1 pandemic were more likely than surgical patients to have experienced recent influenza-like illness or acute respiratory illness, or to have concurrent PCR positive influenza or evidence of influenza A IgA antibodies in sera.

METHODS

Setting, design and participants

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. Cases were patients aged ≥40 years who had experienced 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 myocardial infarction within the past month. These patients were chosen as controls because their admissions were considered unlikely to be influenced by recent influenza-like illness. Cases and controls were 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. The study size was based on numbers of eligible patients hospitalised during the influenza circulation period.

Exposures

The main exposure was recent influenza-like illness, defined as a history of feeling feverish with either cough or sore throat within the last month. We 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 criteria for influenza-like illness. Additional exposures were nasopharyngeal and throat swabs testing positive for influenza by real-time polymerase chain reaction (PCR), presence of IgA antibodies to influenza A in serum samples and self-reported influenza vaccination status.

Data sources and measurement

We used a questionnaire to investigate recent respiratory and influenza-like illness as well as to capture data on demographics, medical history and influenza vaccination status. Information on influenza vaccination status was collected as 'vaccinated this year (from September 2009)', 'vaccinated last year (September 2008 - August 2009)', 'vaccinated 2-5 years ago', 'vaccinated >5 years ago' and 'never vaccinated'. Vaccination status was then re-categorised as a binary variable comprising 'vaccinated this year' and 'unvaccinated', which included all other categories, to recognise that vaccinations in previous seasons would not protect against the new circulating pandemic influenza strain. Medical records were reviewed for details of the current admission and to confirm data on potential confounding factors. Combined nasopharyngeal and throat swabs were taken from each participant, placed in viral transport medium and transported to the laboratory for storage at-80˚C. Samples were tested for the presence of influenza virus RNA using a validated in house real-time PCR with a lower limit of detection of 1 RNA copy per reaction, as previously described[12]. A single serum sample was taken for quantification of IgA antibodies to influenza A as a marker of recent exposure (as IgA levels peak at around 2 weeks after exposure and reach baseline by around 4-6 weeks). Serum samples were centrifuged, frozen at -80˚C and batch tested using a commercially available enzyme-linked immunosorbent assay (EIA) for influenza A IgA (Biosupply UK, cat no. RE56501). Antibody concentrations were initially explored as a continuous 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.

Statistical methods

All analyses were carried out using Stata (*Stata Statistical Software: Release 12. College Station, TX: StataCorp LP*). Baseline comparability between cases and controls was assessed with X^2 tests. 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. We used multivariable logistic regression analysis to investigate associations between recent influenza-like illness or acute respiratory illness exposures and case/ control status, controlling for the frequency-matching factors age-group, gender and month of admission and influenza vaccination status as an a priori confounder (all models) as well as for other potential confounding factors. An additional multivariable model in which influenza vaccination status was the main exposure was generated using the same approach. Potential confounders were examined using a backwards stepwise approach whereby factors independently associated with both exposure and outcome were included in models and likelihood ratio tests used to assess the effect of removing each one sequentially. If p values from likelihood ratio tests were <0.1 then factors were retained in the model.

RESULTS

Characteristics of study participants

134 participants were recruited, who comprised 70 cases from 106 approached (acceptance rate 66%) and 64 controls from 95 approached (acceptance rate 67%). Reasons for non-participation included lack of time, unwillingness to experience additional procedures and feeling tired or unwell. The median age of participants was 63.6 years (IQR 53.3-72.6) and 21% were female. Cases were more likely to be of Asian or Asian British ethnicity (p=0.016), to have a previous history of myocardial infarction (p=0.04) or a family history of myocardial infarction (p<0.001) than controls. Of the 70 patients hospitalised for acute myocardial infarction, 48 (68.5%) met criteria for ST-elevation myocardial infarction, 17 (24.3%)

had a non ST-elevation myocardial infarction and in 5 (7.1%) cases the subtype of myocardial infarction was unspecified. Control patients were admitted with a range of acute non-vascular surgical presentations that included colorectal, urological and orthopaedic conditions.

Table 1

Timing of participants' admissions in relation to national influenza circulation

A comparison of study participants' dates of admission with national rates of GP consultations for influenza-like illness (ILI) based on RCGP surveillance data is shown in figure 1. The peak week for ILI consultations in England was week 43 (ending 25th October 2009) when 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 samples reached 41.2%. Our recruitment period spanned this period of peak influenza circulation.

Figure 1

Recent respiratory and influenza-like illness

17 cases (24.3%) and 12 controls (18.8%) reported respiratory illness in the month preceding hospital admission. 13 illnesses – reported by 10 cases (14.3%) and 3 controls (4.7%) – met criteria for influenza-like illness (defined as feeling feverish with cough and/or sore throat). The most frequently reported category for the timing of respiratory symptom onset was 8-14 days before admission (31.0% of illnesses). 4-7 days was the most frequently reported category for length of illness (37.9% of illnesses). Symptom profiles of participants reporting recent respiratory illness are shown in figure 2. No swabs tested positive for influenza virus nucleic acid. Serum samples were available on 113 of 134 participants (84.3%). There were no significant differences in characteristics of participants with and without missing serum samples (data not shown). 25 cases (43.1%) and 28 controls (50.9%) tested positive for serum

influenza A IgA antibodies. 62% of participants who were seropositive had received influenza vaccination during the study period compared to 31% of seronegative participants. Overall 44.0% of participants were vaccinated. The proportion of participants recruited in each month who were vaccinated increased from 0% in September 2009, to 29.6% in October 2009, 44.8% in November 2009, 55.0% in December 2009, 72.0% in January 2010 and was 50% in February 2010.

Figure 2

Cases were more likely to have reported influenza-like illness than controls – adjusted OR 3.17 (95% confidence interval 0.61-16.47) – as well as other key respiratory illness symptoms, although differences were not statistically significant. Results from this logistic regression analysis are summarised in table 2. There was also a trend towards a protective effect of influenza vaccination against myocardial infarction – adjusted OR 0.46 (95% CI, 0.19-1.12) – after controlling for age-group, gender, month of admission and personal history of AMI.

Table 2

DISCUSSION

The study was supportive of the hypothesis that recent respiratory and in particular influenza-like illnesses occurring during the second wave of the influenza A H1N1 pandemic were more common in patients hospitalised with acute myocardial infarction than with acute surgical conditions. Influenza vaccination was also associated with protection against myocardial infarction, although differences were not statistically significant. While we 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 rates of influenza-like illness remained low [5], especially in age groups typically affected by acute myocardial infarction. The study was therefore underpowered to detect an effect, partly due to limited numbers of infections among participants.

Using self-reported recent respiratory and influenza-like illness as exposures introduced the possibility of reporting or recall bias. Nonetheless 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[13]. 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. We chose to test both nasopharyngeal and throat swabs to increase the sensitivity of virus detection. It was perhaps unsurprising, however, that none of the nasopharyngeal and throat swabs was positive for influenza virus given a) the low rates of infection in this age-group[5] and b) that the majority of viral shedding in influenza occurs in the first 2-3 days after symptom onset[14] whereas most reported respiratory symptoms in study participants occurred 8-14 days before admission. Based on our findings it seems unlikely that any delayed cardiac effect of influenza is linked to ongoing or prolonged virus replication or shedding in the respiratory tract. 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[15] but its effect with the pandemic strain H1N1pdm09 is unclear. It has previously been noted that serological studies carried out during the 2009 influenza pandemic were severely hampered by cross reactivity both with vaccine and with seasonal influenza strains[7].

Previous observational studies using large electronic primary care databases have found an association between GP consultation for acute respiratory infection in the last month and risk of acute myocardial infarction[16-18]. Although studies were conducted over different time periods, they encompassed the effect of varying seasonal influenza strains. Our recent self-controlled case series study using linked primary care and cardiac disease registry data from 3,927 patients also included acute respiratory infection consultations occurring during the first wave of H1N1pdm09 circulation[19]. We found an incidence ratio for acute myocardial infarction of 4.19 (95% confidence interval 3.18-5.53) in the first 1-3 days after acute respiratory infection, with the risk falling to baseline after 28 days[19]. Elderly people and those consulting for an infection judged most likely to be due to influenza were at greatest risk. During the 2009 influenza pandemic people with underlying cardiovascular disease were much more likely to be hospitalised[13] and to die[20] from a range of causes attributable to pandemic influenza. Although most deaths from H1N1pdm09 occurred in younger people, this was partly a function of the age distribution of infections: in the UK the case fatality rate in the elderly was much higher than in younger age-groups[5] but overall numbers of deaths were small as few elderly people were infected.

Various biological mechanisms are proposed to underlie a relationship between influenza or acute respiratory infection and myocardial infarction[21]. Acute respiratory infections may result in a host of acute inflammatory and haemostatic effects leading to systemic inflammation, altered plasma viscosity, coagulability and haemodynamic changes[22] as well as promoting local endothelial dysfunction, coronary inflammation and plaque rupture[23]. Immobility associated with bed-rest and dehydration might potentiate these processes.

In conclusion, this study suggests that recent ILI occurring during the 2009 influenza pandemic was more common in AMI patients. Taken in the context of previous work, this helps to support the hypothesis that, as with other influenza strains, H1N1pdm09 could potentially trigger AMI in vulnerable groups. It is likely, however, that the effect is not specific to influenza and could have also been due to other viruses circulating at the time. The population impact of H1N1pdm09 on rates of hospitalisations and deaths from myocardial infarction is also likely to have been relatively low given the mismatch between the ages of those typically affected by H1N1pdm09 and acute coronary events as well as the relatively mild clinical effects of this influenza strain.

Acknowledgments

We thank all patients who agreed to participate in this study and nurses on the cardiology and surgical wards at the Royal Free Hospital who helped to identify potential participants. We acknowledge Mauli Patel at the Royal Free Virology Laboratory who kindly processed, stored and tested samples.

Contributors

CWG conceived and designed the study, recruited participants, collected and analysed data and wrote the manuscript. AMG, GH, RDR, LS and ACH assisted with study design. AMG provided laboratory resources. CWG, AMG, RDR, LS and ACH interpreted results. AMG, GH, RDR, LS and ACH critically revised the manuscript for intellectual content. LS and ACH provided supervision. All authors approved the final version of the manuscript.

Funding statement

This work was funded by a Medical Research Council Clinical Research Training Fellowship for CWG, grant number G0800689. LS is supported by a Wellcome Trust Senior Research Fellowship in Clinical Science, grant number 098504/Z/12/Z.

Competing interests statement

None declared

Data sharing statement

No additional data are available

References

1. Cox NJ, Subbarao K (1999) Influenza. The Lancet 354: 1277–1282.
2. Warren-Gash C, Smeeth L, Hayward AC (2009) Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. The Lancet Infectious Diseases 9: 601–610.
3. Swine influenza A (H1N1) infection in two children – Southern California, March-April 2009 (2009). MMWR Morb Mortal Wkly Rep 58: 400–402.
4. Outbreak of swine-origin influenza A (H1N1) virus infection – Mexico, March-April 2009 (2009). MMWR Morb Mortal Wkly Rep 58: 467–470.
5. Health Protection Agency (2010) Epidemiological report of pandemic (H1N1) 2009 in the United Kingdom: April 2009 - May 2010.
6. McLean E, Pebody RG, Campbell C, Chamberland M, Hawkins C, et al. (2010) Pandemic (H1N1) 2009 influenza in the UK: clinical and epidemiological findings from the first few hundred (FF100) cases. Epidemiol Infect 138: 1531–1541.
7. Lagacé-Wiens PRS, Rubinstein E, Gumel A (2010) Influenza epidemiology – past, present, and future. Crit Care Med 38: e1–e9.
8. Chacko B, Peter JV, Pichamuthu K, Ramakrishna K, Moorthy M, et al. (2012) Cardiac manifestations in patients with pandemic (H1N1) 2009 virus infection needing intensive care. J Crit Care 27: 106.e1–6.
9. Martin SS, Hollingsworth CL, Norfolk SG, Wolfe CR, Hollingsworth JW. (2010) Reversible cardiac dysfunction associated with pandemic 2009 influenza A (H1N1). Chest 137: 1195-7.
10. Lee N, Chan PKS, Lui GCY, Wong BCK, Sin WWY, et al. (2011) Complications and Outcomes of Pandemic 2009 Influenza A (H1N1) Virus Infection in Hospitalized Adults: How Do They Differ From Those in Seasonal Influenza? J Infect Dis 203: 1739–1747.
11. Dawood FS, Iuliano AD, Reed C, Meltzer MI, Shay DK, et al. (2012) Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. The Lancet Infectious Diseases 12: 687–695.
12. Khanom AB, Velvin C, Hawrami K, Schutten M, Patel M, et al. (2011) Performance of a nurse-led paediatric point of care service for respiratory syncytial virus testing in secondary care. J Infect 62: 52–58.
13. Campbell CNJ, Mytton OT, McLean EM, Rutter PD, Pebody RG, et al. (2010) Hospitalization in two waves of pandemic influenza A(H1N1) in England. Epidemiology & Infection 139: 1560–1569.
14. Lau LLH, Cowling BJ, Fang VJ, Chan K-H, Lau EHY, et al. (2010) Viral shedding and clinical illness in naturally acquired influenza virus infections. J Infect Dis 201: 1509–1516.
15. Rothbarth PH, Groen J, Bohnen AM, De Groot R, Osterhaus AD (1999) Influenza virus serology – a comparative study. J Virol Methods 78: 163–169.
16. Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, et al. (2004) Risk of myocardial infarction and stroke after acute infection or vaccination. New England Journal of Medicine 351: 2611–2618.
17. Clayton TC, Thompson M, Meade TW (2008) Recent respiratory infection and risk of cardiovascular disease: case-control study through a general practice database. Eur Heart J 29: 96–103.
18. Meier CR, Jick SS, Derby LE, Vasilakis C, Jick H, et al. (1998) Acute respiratory-tract infections and risk of first-time acute myocardial infarction. The Lancet 351: 1467–1471.
19. Warren-Gash C, Hayward AC, Hemingway H, Denaxas S, Thomas SL, et al. (2012) Influenza infection and risk of acute myocardial infarction in England and Wales: a CALIBER self-controlled case series study. J Infect Dis 206: 1652–1659.
20. Mytton OT, Rutter PD, Mak M, Stanton E, Sachedina N, et al. (2012) Mortality due to pandemic (H1N1) 2009 influenza in England: a comparison of the first and second waves. Epidemiology & Infection 140: 1533–1541.

21. Bazaz R, Marriott HM, Francis SE, Dockrell DH (2013) Mechanistic links between acute respiratory tract infections and acute coronary syndromes. J Infect 66: 1-17.
22. Harskamp RE, Van Ginkel MW (2008) Acute respiratory tract infections: a potential trigger for the acute coronary syndrome. Annals of Medicine 40: 121–128.
23. Vallance P, Collier J, Bhagat K (1997) Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? The Lancet 349: 1391–1392.

Figure legends

Figure 1 Number of study participants admitted by influenza surveillance week compared to weekly ILI rates per 100,000 based on national RCGP surveillance data

Figure 2 Percentage of cases (n=70) and controls (n=64) reporting various symptoms during respiratory illness

Characteristic	Category	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)	
Admission month	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)	
BMI category	18.5-24.9	20 (30.8)	23 (39.0)	0.41
	25.0-29.9	36 (55.4)	24 (40.7)	
	30.0-39.9	8 (12.3)	10 (17.0)	
	40.0-max	1 (1.5)	2 (3.4)	
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)	
Past medical history	Hypercholesterolaemia*	34 (48.6)	28 (43.8)	0.58
	Diabetes	14 (20.0)	12 (18.8)	0.86
	Hypertension	37 (52.9)	26 (40.6)	0.16
	AMI	14 (20.0)	5 (7.8)	0.04
	Stroke	1 (1.4)	4 (6.3)	0.14
Family history	AMI	43 (61.4)	19 (29.7)	<0.001
	Stroke	5 (7.1)	6 (9.4)	0.64
Influenza vaccination status†	Vaccinated	30 (42.9)	29 (45.3)	0.78

Table 1 Characteristics of study participants (n=134)

*28 cases (40%) and 25 controls (39%) with hypercholesterolaemia reported current statin use ᵼ'Vaccinated' refers to receiving influenza vaccination in the current vaccination year (since September 2009).

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. Muscle ache	8 (11.4)	5 (7.8)	1.52 (0.47-4.92)	2.29 (0.59-8.92)
7. Influenza A IgA antibodies±	25 (46.3)	28 (54.9)	0.71 (0.33-1.53)	0.82 (0.34-2.00)

Table 2 Odds ratios for the association between acute myocardial infarction and various respiratory illness exposure variables, 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 8 equivocal results were excluded, compared to n=134 (70 cases and 64 controls) for all other exposures.

Title Influenza-like illness in acute myocardial infarction patients during the winter wave of the influenza A H1N1 pandemic in London: a case control study

Authors Charlotte Warren-Gash[1], Anna Maria Geretti[2], George Hamilton[3], Roby D. Rakhit[4], Liam Smeeth[5], Andrew C. Hayward[1]

Affiliations

[1]Centre for Infectious Disease Epidemiology, Research Department of Infection & Population Health, UCL, London, UK

[2] Institute of Infection and Global Health, University of Liverpool, UK

[3] Department of Vascular Surgery, Royal Free London Foundation Trust, London, UK

[4] Department of Cardiology, Royal Free London Foundation Trust, London, UK

[5] Department of Non-Communicable Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, UK

Corresponding author

Dr Charlotte Warren-Gash

Research Department of Infection & Population Health

1st Floor, Royal Free Hospital

Rowland Hill Street

London NW3 2PF

Email: c.warren-gash@ucl.ac.uk / charlotte.warren-gash@doctors.org.uk

Tel: 020 7794 0500 x36720 Fax: 020 7794 1224

Word Count Abstract 296, main text 2389 (excluding article summary, tables, figures, legends and refs)

ABSTRACT

Objective: To investigate recent respiratory and influenza-like illnesses in acute myocardial infarction patients compared to patients hospitalised for acute non vascular surgical conditions during the second wave of the 2009 influenza A H1N1 pandemic

Design: Case control study

Setting: Coronary care unit, acute cardiology and acute surgical admissions wards in a major teaching hospital in London, UK

Participants: 134 participants (70 cases and 64 controls) aged ≥40 years hospitalised for acute myocardial infarction and acute surgical conditions between 21/09/2009 and 28/02/2010, frequency-matched for gender, 5-year age-band and admission week

Primary exposure: Influenza-like illness (ILI - defined as feeling feverish with either cough or sore throat) within the last month. **Secondary exposures**: Acute respiratory illness within the last month not meeting ILI criteria; nasopharyngeal and throat swab positive for influenza virus

Results: 29 participants of 134 (21.6%) reported respiratory illness within the last month, of whom 13 (9.7%) had illnesses meeting ILI criteria. The most frequently reported category for timing of respiratory symptom onset was 8-14 days before admission (31.0% of illnesses). Cases were more likely than controls to report ILI – adjusted OR 3.17 (95% confidence interval 0.61-16.47) – as well as other key respiratory symptoms, and were less likely to have received influenza vaccination – adjusted OR 0.46 (95% CI, 0.19-1.12) – although differences were not statistically significant. No swabs were positive for influenza virus.

Conclusions: Point estimates suggested that recent ILI was more common in patients hospitalised with acute myocardial infarction than with acute surgical conditions during the second wave of the influenza A H1N1 pandemic, and influenza vaccination was associated with cardio-protection, although findings were not statistically significant. The study was underpowered, partly because the age groups typically

affected by acute myocardial infarction had low rates of infection with the pandemic influenza strain compared to seasonal influenza.

ARTICLE SUMMARY

Article focus

- Seasonal influenza can trigger cardiovascular complications but the cardiac effects of the 2009 influenza pandemic are less clear.
- We aimed to investigate recent influenza-like illness in patients hospitalised with acute myocardial infarction and surgical conditions during the 2009 influenza pandemic in London.

Key messages

- 14.3% of patients hospitalised with acute myocardial infarction (cases) reported recent influenza-like illness compared to 4.7% of patients hospitalised for acute surgical conditions (controls)
- Cases were more likely than controls to report a range of recent respiratory symptoms and less likely to have received influenza vaccination though differences were not statistically significant
- The median age of cases with acute myocardial infarction was 63.6 years, whereas the majority of people infected with pandemic influenza strain nationally were young

Strengths and limitations

- The study was underpowered to detect an effect, partly due to low infection rates with the pandemic influenza virus in age-groups typically affected by acute myocardial infarction, but it will inform design of future similar studies

INTRODUCTION

Seasonal influenza can trigger cardiovascular complications and deaths in vulnerable populations, especially the elderly and those with underlying medical conditions[1]. Evidence to support the hypothesis that seasonal influenza may trigger acute myocardial infarction comes from a range of observational studies incorporating the effects of different circulating influenza strains and subtypes[2]. 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 [3,4]. 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[5]. Initial evidence from the first wave in the UK suggested that typical illnesses were mild and affected mainly children and young people[6]. The average age of cases increased over subsequent waves of the pandemic[7] but it is unclear how this affected clinical illness profiles. Vaccination coverage did not reach high levels until the post-pandemic season.

There have been reports of myocarditis, myocardial injury and left ventricular systolic dysfunction, which may be reversible, in patients with severe H1N1pdm09[8,9]. It has been suggested that H1N1pdm09 was associated with higher rates of extra-pulmonary complications than seasonal influenza[10] 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[11], but the contribution of myocardial infarction deaths to this figure is unknown.

In this study we aimed to investigate whether patients hospitalised for acute myocardial infarction during the winter wave of the influenza A H1N1 pandemic were more likely than surgical patients to have experienced recent influenza-like illness or acute respiratory illness, or to have concurrent PCR positive influenza or evidence of influenza A IgA antibodies in sera.

METHODS

Setting, design and participants

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. Cases were patients aged ≥40 years who had experienced 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 myocardial infarction within the past month. These patients were chosen as controls because their admissions were considered unlikely to be influenced by recent influenza-like illness. Cases and controls were 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. The study size was based on numbers of eligible patients hospitalised during the influenza circulation period.

Exposures

The main exposure was recent influenza-like illness, defined as a history of feeling feverish with either cough or sore throat within the last month. We 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 criteria for influenza-like illness. Additional exposures were nasopharyngeal and throat swabs testing positive for influenza by real-time polymerase chain reaction (PCR), presence of IgA antibodies to influenza A in serum samples and self-reported influenza vaccination status.

Data sources and measurement

We used a questionnaire to investigate recent respiratory and influenza-like illness as well as to capture data on demographics, medical history and influenza vaccination status. Information on influenza vaccination status was collected as 'vaccinated this year (from September 2009)', 'vaccinated last year (September 2008 - August 2009)', 'vaccinated 2-5 years ago', 'vaccinated >5 years ago' and 'never vaccinated'. Vaccination status was then re-categorised as a binary variable comprising 'vaccinated this year' and 'unvaccinated', which included all other categories, to recognise that vaccinations in previous seasons would not protect against the new circulating pandemic influenza strain. Medical records were reviewed for details of the current admission and to confirm data on potential confounding factors. Combined nasopharyngeal and throat swabs were taken from each participant, placed in viral transport medium and transported to the laboratory for storage at-80˚C. Samples were tested for the presence of influenza virus RNA using a validated in house real-time PCR with a lower limit of detection of 1 RNA copy per reaction, as previously described[12]. A single serum sample was taken for quantification of IgA antibodies to influenza A as a marker of recent exposure (as IgA levels peak at around 2 weeks after exposure and reach baseline by around 4-6 weeks). Serum samples were centrifuged, frozen at -80˚C and batch tested using a commercially available enzyme-linked immunosorbent assay (EIA) for influenza A IgA (Biosupply UK, cat no. RE56501). Antibody concentrations were initially explored as a continuous 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.

Statistical methods

All analyses were carried out using Stata (*Stata Statistical Software: Release 12. College Station, TX: StataCorp LP*). Baseline comparability between cases and controls was assessed with X^2 tests. 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. We used multivariable logistic regression analysis to investigate associations between recent influenza-like illness or acute respiratory illness exposures and case/ control status, controlling for the frequency-matching factors age-group, gender and month of admission and influenza vaccination status as an a priori confounder (all models) as well as for other potential confounding factors. An additional multivariable model in which influenza vaccination status was the main exposure was generated using the same approach. Potential confounders were examined using a backwards stepwise approach whereby factors independently associated with both exposure and outcome were included in models and likelihood ratio tests used to assess the effect of removing each one sequentially. If p values from likelihood ratio tests were <0.1 then factors were retained in the model.

RESULTS

Characteristics of study participants

134 participants were recruited, who comprised 70 cases from 106 approached (acceptance rate 66%) and 64 controls from 95 approached (acceptance rate 67%). Reasons for non-participation included lack of time, unwillingness to experience additional procedures and feeling tired or unwell. The median age of participants was 63.6 years (IQR 53.3-72.6) and 21% were female. Cases were more likely to be of Asian or Asian British ethnicity (p=0.016), to have a previous history of myocardial infarction (p=0.04) or a family history of myocardial infarction (p<0.001) than controls. Of the 70 patients hospitalised for acute myocardial infarction, 48 (68.5%) met criteria for ST-elevation myocardial infarction, 17 (24.3%)

had a non ST-elevation myocardial infarction and in 5 (7.1%) cases the subtype of myocardial infarction was unspecified. Control patients were admitted with a range of acute non-vascular surgical presentations that included colorectal, urological and orthopaedic conditions.

Table 1

Timing of participants' admissions in relation to national influenza circulation

A comparison of study participants' dates of admission with national rates of GP consultations for influenza-like illness (ILI) based on RCGP surveillance data is shown in figure 1. The peak week for ILI consultations in England was week 43 (ending 25th October 2009) when 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 samples reached 41.2%. Our recruitment period spanned this period of peak influenza circulation.

Figure 1

Recent respiratory and influenza-like illness

17 cases (24.3%) and 12 controls (18.8%) reported respiratory illness in the month preceding hospital admission. 13 illnesses – reported by 10 cases (14.3%) and 3 controls (4.7%) – met criteria for influenza-like illness (defined as feeling feverish with cough and/or sore throat). The most frequently reported category for the timing of respiratory symptom onset was 8-14 days before admission (31.0% of illnesses). 4-7 days was the most frequently reported category for length of illness (37.9% of illnesses). Symptom profiles of participants reporting recent respiratory illness are shown in figure 2. No swabs tested positive for influenza virus nucleic acid. Serum samples were available on 113 of 134 participants (84.3%). There were no significant differences in characteristics of participants with and without missing serum samples (data not shown). 25 cases (43.1%) and 28 controls (50.9%) tested positive for serum

influenza A IgA antibodies. 62% of participants who were seropositive had received influenza vaccination during the study period compared to 31% of seronegative participants. Overall 44.0% of participants were vaccinated. The proportion of participants recruited in each month who were vaccinated increased from 0% in September 2009, to 29.6% in October 2009, 44.8% in November 2009, 55.0% in December 2009, 72.0% in January 2010 and was 50% in February 2010.

Figure 2

Cases were more likely to have reported influenza-like illness than controls – adjusted OR 3.17 (95% confidence interval 0.61-16.47) – as well as other key respiratory illness symptoms, although differences were not statistically significant. Results from this logistic regression analysis are summarised in table 2. There was also a trend towards a protective effect of influenza vaccination against myocardial infarction – adjusted OR 0.46 (95% CI, 0.19-1.12) – after controlling for age-group, gender, month of admission and personal history of AMI.

Table 2

DISCUSSION

The study was supportive of the hypothesis that recent respiratory and in particular influenza-like illnesses occurring during the second wave of the influenza A H1N1 pandemic were more common in patients hospitalised with acute myocardial infarction than with acute surgical conditions. Influenza vaccination was also associated with protection against myocardial infarction, although differences were not statistically significant. While we 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 rates of influenza-like illness remained low [5], especially in age groups typically affected by acute myocardial infarction. The study was therefore underpowered to detect an effect, partly due to limited numbers of infections among participants.

Using self-reported recent respiratory and influenza-like illness as exposures introduced the possibility of reporting or recall bias. Nonetheless 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[13]. 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. We chose to test both nasopharyngeal and throat swabs to increase the sensitivity of virus detection. It was perhaps unsurprising, however, that none of the nasopharyngeal and throat swabs was positive for influenza virus given a) the low rates of infection in this age-group[5] and b) that the majority of viral shedding in influenza occurs in the first 2-3 days after symptom onset[14] whereas most reported respiratory symptoms in study participants occurred 8-14 days before admission. Based on our findings it seems unlikely that any delayed cardiac effect of influenza is linked to ongoing or prolonged virus replication or shedding in the respiratory tract. 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[15] but its effect with the pandemic strain H1N1pdm09 is unclear. It has previously been noted that serological studies carried out during the 2009 influenza pandemic were severely hampered by cross reactivity both with vaccine and with seasonal influenza strains[7].

Previous observational studies using large electronic primary care databases have found an association between GP consultation for acute respiratory infection in the last month and risk of acute myocardial infarction[16-18]. Although studies were conducted over different time periods, they encompassed the effect of varying seasonal influenza strains. Our recent self-controlled case series study using linked primary care and cardiac disease registry data from 3,927 patients also included acute respiratory infection consultations occurring during the first wave of H1N1pdm09 circulation[19]. We found an incidence ratio for acute myocardial infarction of 4.19 (95% confidence interval 3.18-5.53) in the first 1-3 days after acute respiratory infection, with the risk falling to baseline after 28 days[19]. Elderly people and those consulting for an infection judged most likely to be due to influenza were at greatest risk. During the 2009 influenza pandemic people with underlying cardiovascular disease were much more likely to be hospitalised[13] and to die[20] from a range of causes attributable to pandemic influenza. Although most deaths from H1N1pdm09 occurred in younger people, this was partly a function of the age distribution of infections: in the UK the case fatality rate in the elderly was much higher than in younger age-groups[5] but overall numbers of deaths were small as few elderly people were infected.

Various biological mechanisms are proposed to underlie a relationship between influenza or acute respiratory infection and myocardial infarction[21]. Acute respiratory infections may result in a host of acute inflammatory and haemostatic effects leading to systemic inflammation, altered plasma viscosity, coagulability and haemodynamic changes[22] as well as promoting local endothelial dysfunction, coronary inflammation and plaque rupture[23]. Immobility associated with bed-rest and dehydration might potentiate these processes.

In conclusion, this study suggests that recent ILI occurring during the 2009 influenza pandemic was more common in AMI patients. Taken in the context of previous work, this helps to support the hypothesis that, as with other influenza strains, H1N1pdm09 could potentially trigger AMI in vulnerable groups. It is likely, however, that the effect is not specific to influenza and could have also been due to other viruses circulating at the time. The population impact of H1N1pdm09 on rates of hospitalisations and deaths from myocardial infarction is also likely to have been relatively low given the mismatch between the ages of those typically affected by H1N1pdm09 and acute coronary events as well as the relatively mild clinical effects of this influenza strain.

Acknowledgments

We thank all patients who agreed to participate in this study and nurses on the cardiology and surgical wards at the Royal Free Hospital who helped to identify potential participants. We acknowledge Mauli Patel at the Royal Free Virology Laboratory who kindly processed, stored and tested samples.

Contributors

CWG conceived and designed the study, recruited participants, collected and analysed data and wrote the manuscript. AMG, GH, RDR, LS and ACH assisted with study design. AMG provided laboratory resources. CWG, AMG, RDR, LS and ACH interpreted results. AMG, GH, RDR, LS and ACH critically revised the manuscript for intellectual content. LS and ACH provided supervision. All authors approved the final version of the manuscript.

Funding statement

This work was funded by a Medical Research Council Clinical Research Training Fellowship for CWG, grant number G0800689. LS is supported by a Wellcome Trust Senior Research Fellowship in Clinical Science, grant number 098504/Z/12/Z.

Competing interests statement

None declared

Data sharing statement

No additional data are available

References

1. Cox NJ, Subbarao K (1999) Influenza. The Lancet 354: 1277–1282.
2. Warren-Gash C, Smeeth L, Hayward AC (2009) Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. The Lancet Infectious Diseases 9: 601–610.
3. Swine influenza A (H1N1) infection in two children – Southern California, March-April 2009 (2009). MMWR Morb Mortal Wkly Rep 58: 400–402.
4. Outbreak of swine-origin influenza A (H1N1) virus infection – Mexico, March-April 2009 (2009). MMWR Morb Mortal Wkly Rep 58: 467–470.
5. Health Protection Agency (2010) Epidemiological report of pandemic (H1N1) 2009 in the United Kingdom: April 2009 - May 2010.
6. McLean E, Pebody RG, Campbell C, Chamberland M, Hawkins C, et al. (2010) Pandemic (H1N1) 2009 influenza in the UK: clinical and epidemiological findings from the first few hundred (FF100) cases. Epidemiol Infect 138: 1531–1541.
7. Lagacé-Wiens PRS, Rubinstein E, Gumel A (2010) Influenza epidemiology – past, present, and future. Crit Care Med 38: e1–e9.
8. Chacko B, Peter JV, Pichamuthu K, Ramakrishna K, Moorthy M, et al. (2012) Cardiac manifestations in patients with pandemic (H1N1) 2009 virus infection needing intensive care. J Crit Care 27: 106.e1–6.
9. Martin SS, Hollingsworth CL, Norfolk SG, Wolfe CR, Hollingsworth JW. (2010) Reversible cardiac dysfunction associated with pandemic 2009 influenza A (H1N1). Chest 137: 1195-7.
10. Lee N, Chan PKS, Lui GCY, Wong BCK, Sin WWY, et al. (2011) Complications and Outcomes of Pandemic 2009 Influenza A (H1N1) Virus Infection in Hospitalized Adults: How Do They Differ From Those in Seasonal Influenza? J Infect Dis 203: 1739–1747.
11. Dawood FS, Iuliano AD, Reed C, Meltzer MI, Shay DK, et al. (2012) Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. The Lancet Infectious Diseases 12: 687–695.
12. Khanom AB, Velvin C, Hawrami K, Schutten M, Patel M, et al. (2011) Performance of a nurse-led paediatric point of care service for respiratory syncytial virus testing in secondary care. J Infect 62: 52–58.
13. Campbell CNJ, Mytton OT, McLean EM, Rutter PD, Pebody RG, et al. (2010) Hospitalization in two waves of pandemic influenza A(H1N1) in England. Epidemiology & Infection 139: 1560–1569.
14. Lau LLH, Cowling BJ, Fang VJ, Chan K-H, Lau EHY, et al. (2010) Viral shedding and clinical illness in naturally acquired influenza virus infections. J Infect Dis 201: 1509–1516.
15. Rothbarth PH, Groen J, Bohnen AM, De Groot R, Osterhaus AD (1999) Influenza virus serology – a comparative study. J Virol Methods 78: 163–169.
16. Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, et al. (2004) Risk of myocardial infarction and stroke after acute infection or vaccination. New England Journal of Medicine 351: 2611–2618.
17. Clayton TC, Thompson M, Meade TW (2008) Recent respiratory infection and risk of cardiovascular disease: case-control study through a general practice database. Eur Heart J 29: 96–103.
18. Meier CR, Jick SS, Derby LE, Vasilakis C, Jick H, et al. (1998) Acute respiratory-tract infections and risk of first-time acute myocardial infarction. The Lancet 351: 1467–1471.
19. Warren-Gash C, Hayward AC, Hemingway H, Denaxas S, Thomas SL, et al. (2012) Influenza infection and risk of acute myocardial infarction in England and Wales: a CALIBER self-controlled case series study. J Infect Dis 206: 1652–1659.
20. Mytton OT, Rutter PD, Mak M, Stanton E, Sachedina N, et al. (2012) Mortality due to pandemic (H1N1) 2009 influenza in England: a comparison of the first and second waves. Epidemiology & Infection 140: 1533–1541.

21. Bazaz R, Marriott HM, Francis SE, Dockrell DH (2013) Mechanistic links between acute respiratory tract infections and acute coronary syndromes. J Infect 66: 1-17.
22. Harskamp RE, Van Ginkel MW (2008) Acute respiratory tract infections: a potential trigger for the acute coronary syndrome. Annals of Medicine 40: 121–128.
23. Vallance P, Collier J, Bhagat K (1997) Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? The Lancet 349: 1391–1392.

Figure legends

Figure 1 Number of study participants admitted by influenza surveillance week compared to weekly ILI rates per 100,000 based on national RCGP surveillance data

Figure 2 Percentage of cases (n=70) and controls (n=64) reporting various symptoms during respiratory illness

Characteristic	Category	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)	
Admission month	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)	
BMI category	18.5-24.9	20 (30.8)	23 (39.0)	0.41
	25.0-29.9	36 (55.4)	24 (40.7)	
	30.0-39.9	8 (12.3)	10 (17.0)	
	40.0-max	1 (1.5)	2 (3.4)	
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)	
Past medical history	Hypercholesterolaemia*	34 (48.6)	28 (43.8)	0.58
	Diabetes	14 (20.0)	12 (18.8)	0.86
	Hypertension	37 (52.9)	26 (40.6)	0.16
	AMI	14 (20.0)	5 (7.8)	0.04
	Stroke	1 (1.4)	4 (6.3)	0.14
Family history	AMI	43 (61.4)	19 (29.7)	<0.001
	Stroke	5 (7.1)	6 (9.4)	0.64
Influenza vaccination status†	Vaccinated	30 (42.9)	29 (45.3)	0.78

Table 1 Characteristics of study participants (n=134)

*28 cases (40%) and 25 controls (39%) with hypercholesterolaemia reported current statin use

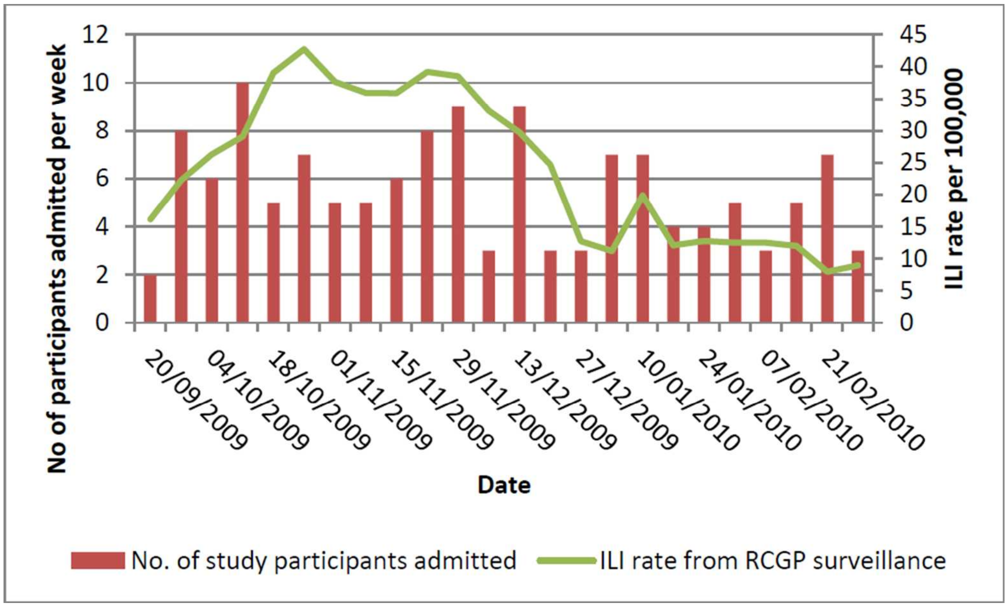
[†]'Vaccinated' refers to receiving influenza vaccination in the current vaccination year (since September 2009).

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. Muscle ache	8 (11.4)	5 (7.8)	1.52 (0.47-4.92)	2.29 (0.59-8.92)
7. Influenza A IgA antibodies[±]	25 (46.3)	28 (54.9)	0.71 (0.33-1.53)	0.82 (0.34-2.00)

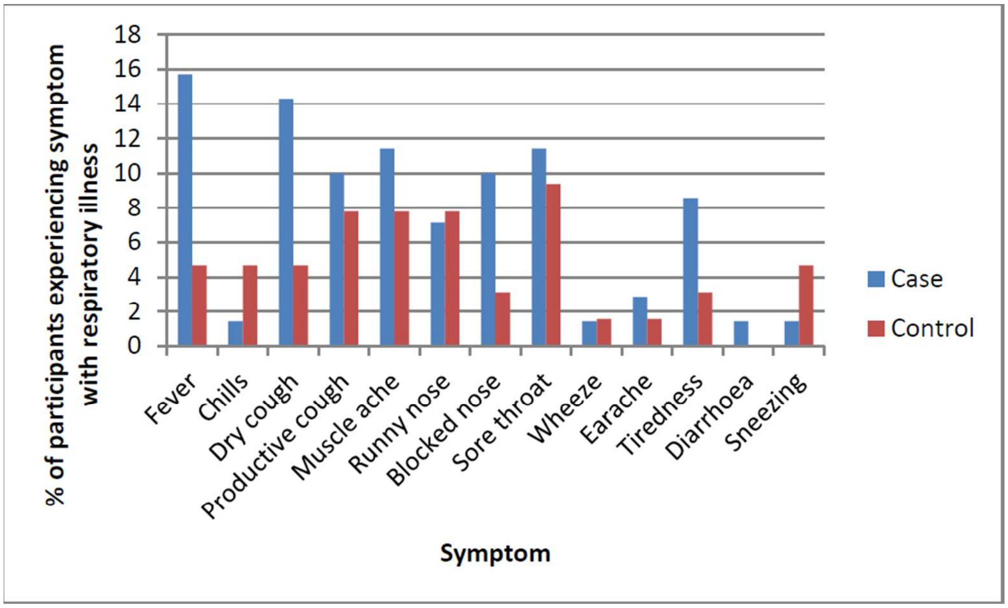
Table 2 Odds ratios for the association between acute myocardial infarction and various respiratory illness exposure variables, unadjusted and adjusted

[*]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 8 equivocal results were excluded, compared to n=134 (70 cases and 64 controls) for all other exposures.



Number of study participants admitted by influenza surveillance week compared to weekly ILI rates per 100,000 based on national RCGP surveillance data
264x158mm (96 x 96 DPI)



Percentage of cases (n=70) and controls (n=64) reporting various symptoms during respiratory illness
264x158mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Reported on page no.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5
		(*b*) For matched studies, give matching criteria and the number of controls per case	Frequency matching criteria given on p5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	Study size based on numbers of eligible patients hospitalised during the influenza circulation period
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how matching of cases and controls was addressed	N/A

		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	Data not systematically collected but reasons for non-participation include lack of time and feeling unwell during admission
		(c) Consider use of a flow diagram	Not done as no follow up with this study design. Fig 1 shows recruitment over time.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1 and p8
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9-10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-002604.R2
Article Type:	Research
Date Submitted by the Author:	27-Mar-2013
Complete List of Authors:	Warren-Gash, Charlotte; UCL, Research Department of Infection & Population Health Geretti, Anna Maria; University of Liverpool, Institute of Infection and Global Health Hamilton, George; Royal Free London Foundation Trust, Department of Vascular Surgery Rakhit, Roby; Royal Free London Foundation Trust, Department of Cardiology Smeeth, Liam; London School of Hygiene and Tropical Medicine, Epidemiology and Population Health Hayward, Andrew; University College London, Infection and Population Health
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Infectious diseases, Cardiovascular medicine
Keywords:	Myocardial infarction < CARDIOLOGY, Epidemiology < INFECTIOUS DISEASES, PUBLIC HEALTH

Title Influenza-like illness in acute myocardial infarction patients during the winter wave of the influenza A H1N1 pandemic in London: a case control study

Authors Charlotte Warren-Gash[1], Anna Maria Geretti[2], George Hamilton[3], Roby D. Rakhit[4], Liam Smeeth[5], Andrew C. Hayward[1]

Affiliations

[1]Centre for Infectious Disease Epidemiology, Research Department of Infection & Population Health, UCL, London, UK

[2] Institute of Infection and Global Health, University of Liverpool, UK

[3] Department of Vascular Surgery, Royal Free London Foundation Trust, London, UK

[4] Department of Cardiology, Royal Free London Foundation Trust, London, UK

[5] Department of Non-Communicable Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, UK

Corresponding author

Dr Charlotte Warren-Gash

Research Department of Infection & Population Health

1st Floor, Royal Free Hospital

Rowland Hill Street

London NW3 2PF

Email: c.warren-gash@ucl.ac.uk / charlotte.warren-gash@doctors.org.uk

Tel: 020 7794 0500 x36720 Fax: 020 7794 1224

Word Count Abstract 296, main text 2505 (excluding article summary, tables, figures, legends and refs)

ABSTRACT

Objective: To investigate recent respiratory and influenza-like illnesses in acute myocardial infarction patients compared to patients hospitalised for acute non vascular surgical conditions during the second wave of the 2009 influenza A H1N1 pandemic

Design: Case control study

Setting: Coronary care unit, acute cardiology and acute surgical admissions wards in a major teaching hospital in London, UK

Participants: 134 participants (70 cases and 64 controls) aged ≥40 years hospitalised for acute myocardial infarction and acute surgical conditions between 21/09/2009 and 28/02/2010, frequency-matched for gender, 5-year age-band and admission week

Primary exposure: Influenza-like illness (ILI - defined as feeling feverish with either cough or sore throat) within the last month. **Secondary exposures**: Acute respiratory illness within the last month not meeting ILI criteria; nasopharyngeal and throat swab positive for influenza virus

Results: 29 participants of 134 (21.6%) reported respiratory illness within the last month, of whom 13 (9.7%) had illnesses meeting ILI criteria. The most frequently reported category for timing of respiratory symptom onset was 8-14 days before admission (31.0% of illnesses). Cases were more likely than controls to report ILI – adjusted OR 3.17 (95% confidence interval 0.61-16.47) – as well as other key respiratory symptoms, and were less likely to have received influenza vaccination – adjusted OR 0.46 (95% CI, 0.19-1.12) – although differences were not statistically significant. No swabs were positive for influenza virus.

Conclusions: Point estimates suggested that recent ILI was more common in patients hospitalised with acute myocardial infarction than with acute surgical conditions during the second wave of the influenza A H1N1 pandemic, and influenza vaccination was associated with cardio-protection, although findings were not statistically significant. The study was underpowered, partly because the age groups typically

affected by acute myocardial infarction had low rates of infection with the pandemic influenza strain compared to seasonal influenza.

ARTICLE SUMMARY

Article focus

- Seasonal influenza can trigger cardiovascular complications but the cardiac effects of the 2009 influenza pandemic are less clear.
- We aimed to investigate recent influenza-like illness in patients hospitalised with acute myocardial infarction and surgical conditions during the 2009 influenza pandemic in London.

Key messages

- 14.3% of patients hospitalised with acute myocardial infarction (cases) reported recent influenza-like illness compared to 4.7% of patients hospitalised for acute surgical conditions (controls)
- Cases were more likely than controls to report a range of recent respiratory symptoms and less likely to have received influenza vaccination though differences were not statistically significant
- The median age of cases with acute myocardial infarction was 63.6 years, whereas the majority of people infected with pandemic influenza strain nationally were young

Strengths and limitations

- The study was underpowered to detect an effect, partly due to low infection rates with the pandemic influenza virus in age-groups typically affected by acute myocardial infarction, but it will inform design of future similar studies

INTRODUCTION

Seasonal influenza can trigger cardiovascular complications and deaths in vulnerable populations, especially the elderly and those with underlying medical conditions[1]. Evidence to support the hypothesis that seasonal influenza may trigger acute myocardial infarction comes from a range of observational studies incorporating the effects of different circulating influenza strains and subtypes[2]. 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 [3,4]. 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[5]. Initial evidence from the first wave in the UK suggested that typical illnesses were mild and affected mainly children and young people[6]. The average age of cases increased over subsequent waves of the pandemic[7] but it is unclear how this affected clinical illness profiles. Vaccination coverage did not reach high levels until the post-pandemic season.

There have been reports of myocarditis, myocardial injury and left ventricular systolic dysfunction, which may be reversible, in patients with severe H1N1pdm09[8,9]. It has been suggested that H1N1pdm09 was associated with higher rates of extra-pulmonary complications than seasonal influenza[10] 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[11], but the contribution of myocardial infarction deaths to this figure is unknown.

In this study we aimed to investigate whether patients hospitalised for acute myocardial infarction during the winter wave of the influenza A H1N1 pandemic were more likely than surgical patients to have experienced recent influenza-like illness or acute respiratory illness, or to have concurrent PCR positive influenza or evidence of influenza A IgA antibodies in sera.

METHODS

Setting, design and participants

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. Cases were patients aged ≥40 years who had experienced 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 myocardial infarction within the past month. These patients were chosen as controls because their admissions were considered unlikely to be influenced by recent influenza-like illness. Cases and controls were 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. The study size was based on numbers of eligible patients hospitalised during the influenza circulation period.

Exposures

The main exposure was recent influenza-like illness, defined as a history of feeling feverish with either cough or sore throat within the last month. We 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 criteria for influenza-like illness. Additional exposures were nasopharyngeal and throat swabs testing positive for influenza by real-time polymerase chain reaction (PCR), presence of IgA antibodies to influenza A in serum samples and self-reported influenza vaccination status.

Data sources and measurement

We used a questionnaire to investigate recent respiratory and influenza-like illness as well as to capture data on demographics, medical history and influenza vaccination status. Information on influenza vaccination status was collected as 'vaccinated this year (from September 2009)', 'vaccinated last year (September 2008 - August 2009)', 'vaccinated 2-5 years ago', 'vaccinated >5 years ago' and 'never vaccinated'. Vaccination status was then re-categorised as a binary variable comprising 'vaccinated this year' and 'unvaccinated', which included all other categories, to recognise that vaccinations in previous seasons would not protect against the new circulating pandemic influenza strain. Medical records were reviewed for details of the current admission and to confirm data on potential confounding factors. Combined nasopharyngeal and throat swabs were taken from each participant, placed in viral transport medium and transported to the laboratory for storage at-80˚C. Samples were tested for the presence of influenza virus RNA using a validated in house real-time PCR with a lower limit of detection of 1 RNA copy per reaction, as previously described[12]. A single serum sample was taken for quantification of IgA antibodies to influenza A as a marker of recent exposure (as IgA levels peak at around 2 weeks after exposure and reach baseline by around 4-6 weeks). Serum samples were centrifuged, frozen at -80˚C and batch tested using a commercially available enzyme-linked immunosorbent assay (EIA) for influenza A IgA (Biosupply UK, cat no. RE56501). Antibody concentrations were initially explored as a continuous 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.

Statistical methods

All analyses were carried out using Stata (*Stata Statistical Software: Release 12. College Station, TX: StataCorp LP*). Baseline comparability between cases and controls was assessed with X^2 tests. 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. We used multivariable logistic regression analysis to investigate associations between recent influenza-like illness or acute respiratory illness exposures and case/ control status, controlling for the frequency-matching factors age-group, gender and month of admission and influenza vaccination status as an a priori confounder (all models) as well as for other potential confounding factors. An additional multivariable model in which influenza vaccination status was the main exposure was generated using the same approach. Potential confounders were examined using a backwards stepwise approach whereby factors independently associated with both exposure and outcome were included in models and likelihood ratio tests used to assess the effect of removing each one sequentially. If p values from likelihood ratio tests were <0.1 then factors were retained in the model.

RESULTS

Characteristics of study participants

134 participants were recruited, who comprised 70 cases from 106 approached (acceptance rate 66%) and 64 controls from 95 approached (acceptance rate 67%). Reasons for non-participation included lack of time, unwillingness to experience additional procedures and feeling tired or unwell. The median age of participants was 63.6 years (IQR 53.3-72.6) and 21% were female. Cases were more likely to be of Asian or Asian British ethnicity (p=0.016), to have a previous history of myocardial infarction (p=0.04) or a family history of myocardial infarction (p<0.001) than controls. Of the 70 patients hospitalised for acute myocardial infarction, 48 (68.5%) met criteria for ST-elevation myocardial infarction, 17 (24.3%)

had a non ST-elevation myocardial infarction and in 5 (7.1%) cases the subtype of myocardial infarction was unspecified. Control patients were admitted with a range of acute non-vascular surgical presentations that included colorectal, urological and orthopaedic conditions.

Table 1

Timing of participants' admissions in relation to national influenza circulation

A comparison of study participants' dates of admission with national rates of GP consultations for influenza-like illness (ILI) based on RCGP surveillance data is shown in figure 1. The peak week for ILI consultations in England was week 43 (ending 25th October 2009) when 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 samples reached 41.2%. Our recruitment period spanned this period of peak influenza circulation.

Figure 1

Recent respiratory and influenza-like illness

17 cases (24.3%) and 12 controls (18.8%) reported respiratory illness in the month preceding hospital admission. 13 illnesses – reported by 10 cases (14.3%) and 3 controls (4.7%) – met criteria for influenza-like illness (defined as feeling feverish with cough and/or sore throat). The most frequently reported category for the timing of respiratory symptom onset was 8-14 days before admission (31.0% of illnesses). 4-7 days was the most frequently reported category for length of illness (37.9% of illnesses). Symptom profiles of participants reporting recent respiratory illness are shown in figure 2. No swabs tested positive for influenza virus nucleic acid. Serum samples were available on 113 of 134 participants (84.3%). There were no significant differences in characteristics of participants with and without missing serum samples (data not shown). 25 cases (43.1%) and 28 controls (50.9%) tested positive for serum

influenza A IgA antibodies. 62% of participants who were seropositive had received influenza vaccination during the study period compared to 31% of seronegative participants. Overall 44.0% of participants were vaccinated. The proportion of participants recruited in each month who were vaccinated increased from 0% in September 2009, to 29.6% in October 2009, 44.8% in November 2009, 55.0% in December 2009, 72.0% in January 2010 and was 50% in February 2010.

Figure 2

Cases were more likely to have reported influenza-like illness than controls – adjusted OR 3.17 (95% confidence interval 0.61-16.47) – as well as other key respiratory illness symptoms, although differences were not statistically significant. Results from this logistic regression analysis are summarised in table 2. There was also a trend towards a protective effect of influenza vaccination against myocardial infarction – adjusted OR 0.46 (95% CI, 0.19-1.12) – after controlling for age-group, gender, month of admission and personal history of AMI.

Table 2

DISCUSSION

The study was supportive of the hypothesis that recent respiratory and in particular influenza-like illnesses occurring during the second wave of the influenza A H1N1 pandemic were more common in patients hospitalised with acute myocardial infarction than with acute surgical conditions. Influenza vaccination was also associated with protection against myocardial infarction, although differences were not statistically significant. While we 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 rates of influenza-like illness remained low [5], especially in age groups typically affected by acute myocardial infarction. The study was therefore underpowered to detect an effect, partly due to limited numbers of infections among participants.

Using self-reported recent respiratory and influenza-like illness as exposures introduced the possibility of reporting or recall bias. Nonetheless 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[13]. 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. We chose to test both nasopharyngeal and throat swabs to increase the sensitivity of virus detection. It was perhaps unsurprising, however, that none of the nasopharyngeal and throat swabs was positive for influenza virus given a) the low rates of infection in this age-group[5] and b) that the majority of viral shedding in influenza occurs in the first 2-3 days after symptom onset[14] whereas most reported respiratory symptoms in study participants occurred 8-14 days before admission. Based on our findings it seems unlikely that any delayed cardiac effect of influenza is linked to ongoing or prolonged virus replication or shedding in the respiratory tract. 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[15] but its effect with the pandemic strain H1N1pdm09 is unclear. It has previously been noted that serological studies carried out during the 2009 influenza pandemic were severely hampered by cross reactivity both with vaccine and with seasonal influenza strains[7].

Previous observational studies using large electronic primary care databases have found an association between GP consultation for acute respiratory infection in the last month and risk of acute myocardial infarction[16-18]. Although studies were conducted over different time periods, they encompassed the effect of varying seasonal influenza strains. In the present study, we controlled for important potential confounders such as influenza vaccination status, and showed that statin use was equally prevalent in cases and controls. We did not, however, have complete data on other drugs that have been hypothesised to have immune-regulatory effects (such as ACE inhibitors ,angiotensin receptor blockers (ARBs), metformin, glitazones and fibrates). If these agents reduce the likelihood of people experiencing ILI and were more commonly used in cases than controls they could potentially have confounded the relationship between ILI and AMI. It was reassuring, however, that our results were consistent with those obtained in our recent self-controlled case series study - a design that implicitly controls for fixed confounders. In this study we used linked primary care and cardiac disease registry records from 3,927 patients from 2003-2009, which also included acute respiratory infection consultations occurring during the first wave of H1N1pdm09 circulation[19]. We found an incidence ratio for acute myocardial infarction of 4.19 (95% confidence interval 3.18-5.53) in the first 1-3 days after acute respiratory infection, with the risk falling to baseline after 28 days[19]. Elderly people and those consulting for an infection judged most likely to be due to influenza were at greatest risk. During the 2009 influenza pandemic people with underlying cardiovascular disease were much more likely to be hospitalised[13] and to die[20] from a range of causes attributable to pandemic influenza. Although most deaths from H1N1pdm09 occurred in younger people, this was partly a function of the age distribution of infections: in the UK the case fatality rate in the elderly was much higher than in younger age-groups[5] but overall numbers of deaths were small as few elderly people were infected.

Various biological mechanisms are proposed to underlie a relationship between influenza or acute respiratory infection and myocardial infarction[21]. Acute respiratory infections may result in a host of acute inflammatory and haemostatic effects leading to systemic inflammation, altered plasma viscosity, coagulability and haemodynamic changes[22] as well as promoting local endothelial dysfunction, coronary inflammation and plaque rupture[23]. Immobility associated with bed-rest and dehydration might potentiate these processes.

In conclusion, this study suggests that recent ILI occurring during the 2009 influenza pandemic was more common in AMI patients. Taken in the context of previous work, this helps to support the hypothesis that, as with other influenza strains, H1N1pdm09 could potentially trigger AMI in vulnerable groups. It is likely, however, that the effect is not specific to influenza and could have also been due to other viruses circulating at the time. The population impact of H1N1pdm09 on rates of hospitalisations and deaths from myocardial infarction is also likely to have been relatively low given the mismatch between the ages of those typically affected by H1N1pdm09 and acute coronary events as well as the relatively mild clinical effects of this influenza strain.

Acknowledgments

We thank all patients who agreed to participate in this study and nurses on the cardiology and surgical wards at the Royal Free Hospital who helped to identify potential participants. We acknowledge Mauli Patel at the Royal Free Virology Laboratory who kindly processed, stored and tested samples.

Contributors

CWG conceived and designed the study, recruited participants, collected and analysed data and wrote the manuscript. AMG, GH, RDR, LS and ACH assisted with study design. AMG provided laboratory resources. CWG, AMG, RDR, LS and ACH interpreted results. AMG, GH, RDR, LS and ACH critically revised the manuscript for intellectual content. LS and ACH provided supervision. All authors approved the final version of the manuscript.

Funding statement

This work was funded by a Medical Research Council Clinical Research Training Fellowship for CWG, grant number G0800689. LS is supported by a Wellcome Trust Senior Research Fellowship in Clinical Science, grant number 098504/Z/12/Z.

Competing interests statement

None declared

Data sharing statement

No additional data are available

References

1. Cox NJ, Subbarao K (1999) Influenza. The Lancet 354: 1277–1282.
2. Warren-Gash C, Smeeth L, Hayward AC (2009) Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. The Lancet Infectious Diseases 9: 601–610.
3. Swine influenza A (H1N1) infection in two children – Southern California, March-April 2009 (2009). MMWR Morb Mortal Wkly Rep 58: 400–402.
4. Outbreak of swine-origin influenza A (H1N1) virus infection – Mexico, March-April 2009 (2009). MMWR Morb Mortal Wkly Rep 58: 467–470.
5. Health Protection Agency (2010) Epidemiological report of pandemic (H1N1) 2009 in the United Kingdom: April 2009 - May 2010.
6. McLean E, Pebody RG, Campbell C, et al. (2010) Pandemic (H1N1) 2009 influenza in the UK: clinical and epidemiological findings from the first few hundred (FF100) cases. Epidemiol Infect 138: 1531–1541.
7. Lagacé-Wiens PRS, Rubinstein E, Gumel A (2010) Influenza epidemiology – past, present, and future. Crit Care Med 38: e1–e9.
8. Chacko B, Peter JV, Pichamuthu K, et al. (2012) Cardiac manifestations in patients with pandemic (H1N1) 2009 virus infection needing intensive care. J Crit Care 27: 106.e1–6.
9. Martin SS, Hollingsworth CL, Norfolk SG, et al. (2010) Reversible cardiac dysfunction associated with pandemic 2009 influenza A (H1N1). Chest 137: 1195-7.
10. Lee N, Chan PKS, Lui GCY, et al. (2011) Complications and Outcomes of Pandemic 2009 Influenza A (H1N1) Virus Infection in Hospitalized Adults: How Do They Differ From Those in Seasonal Influenza? J Infect Dis 203: 1739–1747.
11. Dawood FS, Iuliano AD, Reed C, et al. (2012) Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. The Lancet Infectious Diseases 12: 687–695.

12. Khanom AB, Velvin C, Hawrami K, et al. (2011) Performance of a nurse-led paediatric point of care service for respiratory syncytial virus testing in secondary care. J Infect 62: 52–58.
13. Campbell CNJ, Mytton OT, McLean EM, et al. (2010) Hospitalization in two waves of pandemic influenza A(H1N1) in England. Epidemiology & Infection 139: 1560–1569.
14. Lau LLH, Cowling BJ, Fang VJ, et al. (2010) Viral shedding and clinical illness in naturally acquired influenza virus infections. J Infect Dis 201: 1509–1516.
15. Rothbarth PH, Groen J, Bohnen AM, et al. J Virol Methods 78: 163–169.
16. Smeeth L, Thomas SL, Hall AJ, et al. (2004) Risk of myocardial infarction and stroke after acute infection or vaccination. New England Journal of Medicine 351: 2611–2618.
17. Clayton TC, Thompson M, Meade TW (2008) Recent respiratory infection and risk of cardiovascular disease: case-control study through a general practice database. Eur Heart J 29: 96–103.
18. Meier CR, Jick SS, Derby LE, et al. (1998) Acute respiratory-tract infections and risk of first-time acute myocardial infarction. The Lancet 351: 1467–1471.
19. Warren-Gash C, Hayward AC, Hemingway H, et al. (2012) Influenza infection and risk of acute myocardial infarction in England and Wales: a CALIBER self-controlled case series study. J Infect Dis 206: 1652–1659.
20. Mytton OT, Rutter PD, Mak M, et al. (2012) Mortality due to pandemic (H1N1) 2009 influenza in England: a comparison of the first and second waves. Epidemiology & Infection 140: 1533–1541.
21. Bazaz R, Marriott HM, Francis SE, et al. (2013) Mechanistic links between acute respiratory tract infections and acute coronary syndromes. J Infect 66: 1-17.
22. Harskamp RE, Van Ginkel MW (2008) Acute respiratory tract infections: a potential trigger for the acute coronary syndrome. Annals of Medicine 40: 121–128.
23. Vallance P, Collier J, Bhagat K (1997) Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? The Lancet 349: 1391–1392.

Figure legends

Figure 1 Number of study participants admitted by influenza surveillance week compared to weekly ILI rates per 100,000 based on national RCGP surveillance data

Figure 2 Percentage of cases (n=70) and controls (n=64) reporting various symptoms during respiratory illness

Characteristic	Category	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)	
Admission month	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)	
BMI category	18.5-24.9	20 (30.8)	23 (39.0)	0.41
	25.0-29.9	36 (55.4)	24 (40.7)	
	30.0-39.9	8 (12.3)	10 (17.0)	
	40.0-max	1 (1.5)	2 (3.4)	
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)	
Past medical history	Hypercholesterolaemia*	34 (48.6)	28 (43.8)	0.58
	Diabetes	14 (20.0)	12 (18.8)	0.86
	Hypertension	37 (52.9)	26 (40.6)	0.16
	AMI	14 (20.0)	5 (7.8)	0.04
	Stroke	1 (1.4)	4 (6.3)	0.14
Family history	AMI	43 (61.4)	19 (29.7)	<0.001
	Stroke	5 (7.1)	6 (9.4)	0.64
Influenza vaccination status†	Vaccinated	30 (42.9)	29 (45.3)	0.78

Table 1 Characteristics of study participants (n=134)

*28 cases (40%) and 25 controls (39%) with hypercholesterolaemia reported current statin use

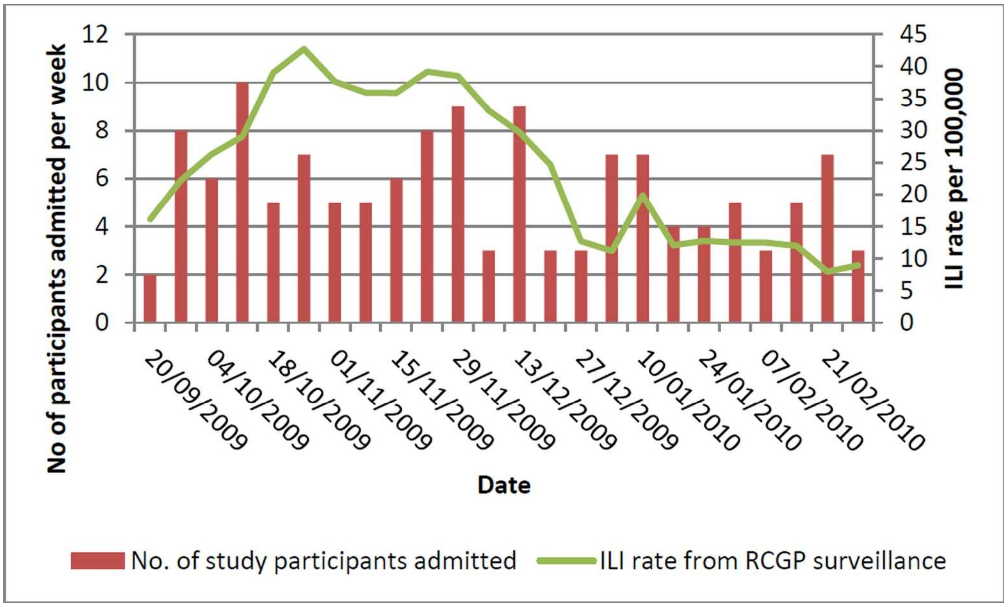
†'Vaccinated' refers to receiving influenza vaccination in the current vaccination year (since September 2009).

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. Muscle ache	8 (11.4)	5 (7.8)	1.52 (0.47-4.92)	2.29 (0.59-8.92)
7. Influenza A IgA antibodies±	25 (46.3)	28 (54.9)	0.71 (0.33-1.53)	0.82 (0.34-2.00)

Table 2 Odds ratios for the association between acute myocardial infarction and various respiratory illness exposure variables, 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 8 equivocal results were excluded, compared to n=134 (70 cases and 64 controls) for all other exposures.



Number of study participants admitted by influenza surveillance week compared to weekly ILI rates per 100,000 based on national RCGP surveillance data
150x90mm (300 x 300 DPI)

Title Influenza-like illness in acute myocardial infarction patients during the winter wave of the influenza A H1N1 pandemic in London: a case control study

Authors Charlotte Warren-Gash[1], Anna Maria Geretti[2], George Hamilton[3], Roby D. Rakhit[4], Liam Smeeth[5], Andrew C. Hayward[1]

Affiliations

[1]Centre for Infectious Disease Epidemiology, Research Department of Infection & Population Health, UCL, London, UK

[2] Institute of Infection and Global Health, University of Liverpool, UK

[3] Department of Vascular Surgery, Royal Free London Foundation Trust, London, UK

[4] Department of Cardiology, Royal Free London Foundation Trust, London, UK

[5] Department of Non-Communicable Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, UK

Corresponding author

Dr Charlotte Warren-Gash

Research Department of Infection & Population Health

1st Floor, Royal Free Hospital

Rowland Hill Street

London NW3 2PF

Email: c.warren-gash@ucl.ac.uk / charlotte.warren-gash@doctors.org.uk

Tel: 020 7794 0500 x36720 Fax: 020 7794 1224

Word Count Abstract 296, main text 2505 (excluding article summary, tables, figures, legends and refs)

ABSTRACT

Objective: To investigate recent respiratory and influenza-like illnesses in acute myocardial infarction patients compared to patients hospitalised for acute non vascular surgical conditions during the second wave of the 2009 influenza A H1N1 pandemic

Design: Case control study

Setting: Coronary care unit, acute cardiology and acute surgical admissions wards in a major teaching hospital in London, UK

Participants: 134 participants (70 cases and 64 controls) aged ≥40 years hospitalised for acute myocardial infarction and acute surgical conditions between 21/09/2009 and 28/02/2010, frequency-matched for gender, 5-year age-band and admission week

Primary exposure: Influenza-like illness (ILI - defined as feeling feverish with either cough or sore throat) within the last month. **Secondary exposures**: Acute respiratory illness within the last month not meeting ILI criteria; nasopharyngeal and throat swab positive for influenza virus

Results: 29 participants of 134 (21.6%) reported respiratory illness within the last month, of whom 13 (9.7%) had illnesses meeting ILI criteria. The most frequently reported category for timing of respiratory symptom onset was 8-14 days before admission (31.0% of illnesses). Cases were more likely than controls to report ILI – adjusted OR 3.17 (95% confidence interval 0.61-16.47) – as well as other key respiratory symptoms, and were less likely to have received influenza vaccination – adjusted OR 0.46 (95% CI, 0.19-1.12) – although differences were not statistically significant. No swabs were positive for influenza virus.

Conclusions: Point estimates suggested that recent ILI was more common in patients hospitalised with acute myocardial infarction than with acute surgical conditions during the second wave of the influenza A H1N1 pandemic, and influenza vaccination was associated with cardio-protection, although findings were not statistically significant. The study was underpowered, partly because the age groups typically

affected by acute myocardial infarction had low rates of infection with the pandemic influenza strain compared to seasonal influenza.

ARTICLE SUMMARY

Article focus

- Seasonal influenza can trigger cardiovascular complications but the cardiac effects of the 2009 influenza pandemic are less clear.
- We aimed to investigate recent influenza-like illness in patients hospitalised with acute myocardial infarction and surgical conditions during the 2009 influenza pandemic in London.

Key messages

- 14.3% of patients hospitalised with acute myocardial infarction (cases) reported recent influenza-like illness compared to 4.7% of patients hospitalised for acute surgical conditions (controls)
- Cases were more likely than controls to report a range of recent respiratory symptoms and less likely to have received influenza vaccination though differences were not statistically significant
- The median age of cases with acute myocardial infarction was 63.6 years, whereas the majority of people infected with pandemic influenza strain nationally were young

Strengths and limitations

- The study was underpowered to detect an effect, partly due to low infection rates with the pandemic influenza virus in age-groups typically affected by acute myocardial infarction, but it will inform design of future similar studies

INTRODUCTION

Seasonal influenza can trigger cardiovascular complications and deaths in vulnerable populations, especially the elderly and those with underlying medical conditions[1]. Evidence to support the hypothesis that seasonal influenza may trigger acute myocardial infarction comes from a range of observational studies incorporating the effects of different circulating influenza strains and subtypes[2]. 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 [3,4]. 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[5]. Initial evidence from the first wave in the UK suggested that typical illnesses were mild and affected mainly children and young people[6]. The average age of cases increased over subsequent waves of the pandemic[7] but it is unclear how this affected clinical illness profiles. Vaccination coverage did not reach high levels until the post-pandemic season.

There have been reports of myocarditis, myocardial injury and left ventricular systolic dysfunction, which may be reversible, in patients with severe H1N1pdm09[8,9]. It has been suggested that H1N1pdm09 was associated with higher rates of extra-pulmonary complications than seasonal influenza[10] 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[11], but the contribution of myocardial infarction deaths to this figure is unknown.

In this study we aimed to investigate whether patients hospitalised for acute myocardial infarction during the winter wave of the influenza A H1N1 pandemic were more likely than surgical patients to have experienced recent influenza-like illness or acute respiratory illness, or to have concurrent PCR positive influenza or evidence of influenza A IgA antibodies in sera.

METHODS

Setting, design and participants

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. Cases were patients aged ≥40 years who had experienced 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 myocardial infarction within the past month. These patients were chosen as controls because their admissions were considered unlikely to be influenced by recent influenza-like illness. Cases and controls were 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. The study size was based on numbers of eligible patients hospitalised during the influenza circulation period.

Exposures

The main exposure was recent influenza-like illness, defined as a history of feeling feverish with either cough or sore throat within the last month. We 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 criteria for influenza-like illness. Additional exposures were nasopharyngeal and throat swabs testing positive for influenza by real-time polymerase chain reaction (PCR), presence of IgA antibodies to influenza A in serum samples and self-reported influenza vaccination status.

Data sources and measurement

We used a questionnaire to investigate recent respiratory and influenza-like illness as well as to capture data on demographics, medical history and influenza vaccination status. Information on influenza vaccination status was collected as 'vaccinated this year (from September 2009)', 'vaccinated last year (September 2008 - August 2009)', 'vaccinated 2-5 years ago', 'vaccinated >5 years ago' and 'never vaccinated'. Vaccination status was then re-categorised as a binary variable comprising 'vaccinated this year' and 'unvaccinated', which included all other categories, to recognise that vaccinations in previous seasons would not protect against the new circulating pandemic influenza strain. Medical records were reviewed for details of the current admission and to confirm data on potential confounding factors. Combined nasopharyngeal and throat swabs were taken from each participant, placed in viral transport medium and transported to the laboratory for storage at-80˚C. Samples were tested for the presence of influenza virus RNA using a validated in house real-time PCR with a lower limit of detection of 1 RNA copy per reaction, as previously described[12]. A single serum sample was taken for quantification of IgA antibodies to influenza A as a marker of recent exposure (as IgA levels peak at around 2 weeks after exposure and reach baseline by around 4-6 weeks). Serum samples were centrifuged, frozen at -80˚C and batch tested using a commercially available enzyme-linked immunosorbent assay (EIA) for influenza A IgA (Biosupply UK, cat no. RE56501). Antibody concentrations were initially explored as a continuous 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.

Statistical methods

All analyses were carried out using Stata (*Stata Statistical Software: Release 12. College Station, TX: StataCorp LP*). Baseline comparability between cases and controls was assessed with X^2 tests. 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. We used multivariable logistic regression analysis to investigate associations between recent influenza-like illness or acute respiratory illness exposures and case/ control status, controlling for the frequency-matching factors age-group, gender and month of admission and influenza vaccination status as an a priori confounder (all models) as well as for other potential confounding factors. An additional multivariable model in which influenza vaccination status was the main exposure was generated using the same approach. Potential confounders were examined using a backwards stepwise approach whereby factors independently associated with both exposure and outcome were included in models and likelihood ratio tests used to assess the effect of removing each one sequentially. If p values from likelihood ratio tests were <0.1 then factors were retained in the model.

RESULTS

Characteristics of study participants

134 participants were recruited, who comprised 70 cases from 106 approached (acceptance rate 66%) and 64 controls from 95 approached (acceptance rate 67%). Reasons for non-participation included lack of time, unwillingness to experience additional procedures and feeling tired or unwell. The median age of participants was 63.6 years (IQR 53.3-72.6) and 21% were female. Cases were more likely to be of Asian or Asian British ethnicity (p=0.016), to have a previous history of myocardial infarction (p=0.04) or a family history of myocardial infarction (p<0.001) than controls. Of the 70 patients hospitalised for acute myocardial infarction, 48 (68.5%) met criteria for ST-elevation myocardial infarction, 17 (24.3%)

had a non ST-elevation myocardial infarction and in 5 (7.1%) cases the subtype of myocardial infarction was unspecified. Control patients were admitted with a range of acute non-vascular surgical presentations that included colorectal, urological and orthopaedic conditions.

Table 1

Timing of participants' admissions in relation to national influenza circulation

A comparison of study participants' dates of admission with national rates of GP consultations for influenza-like illness (ILI) based on RCGP surveillance data is shown in figure 1. The peak week for ILI consultations in England was week 43 (ending 25th October 2009) when 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 samples reached 41.2%. Our recruitment period spanned this period of peak influenza circulation.

Figure 1

Recent respiratory and influenza-like illness

17 cases (24.3%) and 12 controls (18.8%) reported respiratory illness in the month preceding hospital admission. 13 illnesses – reported by 10 cases (14.3%) and 3 controls (4.7%) – met criteria for influenza-like illness (defined as feeling feverish with cough and/or sore throat). The most frequently reported category for the timing of respiratory symptom onset was 8-14 days before admission (31.0% of illnesses). 4-7 days was the most frequently reported category for length of illness (37.9% of illnesses). Symptom profiles of participants reporting recent respiratory illness are shown in figure 2. No swabs tested positive for influenza virus nucleic acid. Serum samples were available on 113 of 134 participants (84.3%). There were no significant differences in characteristics of participants with and without missing serum samples (data not shown). 25 cases (43.1%) and 28 controls (50.9%) tested positive for serum

influenza A IgA antibodies. 62% of participants who were seropositive had received influenza vaccination during the study period compared to 31% of seronegative participants. Overall 44.0% of participants were vaccinated. The proportion of participants recruited in each month who were vaccinated increased from 0% in September 2009, to 29.6% in October 2009, 44.8% in November 2009, 55.0% in December 2009, 72.0% in January 2010 and was 50% in February 2010.

Figure 2

Cases were more likely to have reported influenza-like illness than controls – adjusted OR 3.17 (95% confidence interval 0.61-16.47) – as well as other key respiratory illness symptoms, although differences were not statistically significant. Results from this logistic regression analysis are summarised in table 2. There was also a trend towards a protective effect of influenza vaccination against myocardial infarction – adjusted OR 0.46 (95% CI, 0.19-1.12) – after controlling for age-group, gender, month of admission and personal history of AMI.

Table 2

DISCUSSION

The study was supportive of the hypothesis that recent respiratory and in particular influenza-like illnesses occurring during the second wave of the influenza A H1N1 pandemic were more common in patients hospitalised with acute myocardial infarction than with acute surgical conditions. Influenza vaccination was also associated with protection against myocardial infarction, although differences were not statistically significant. While we 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 rates of influenza-like illness remained low [5], especially in age groups typically affected by acute myocardial infarction. The study was therefore underpowered to detect an effect, partly due to limited numbers of infections among participants.

Using self-reported recent respiratory and influenza-like illness as exposures introduced the possibility of reporting or recall bias. Nonetheless 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[13]. 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. We chose to test both nasopharyngeal and throat swabs to increase the sensitivity of virus detection. It was perhaps unsurprising, however, that none of the nasopharyngeal and throat swabs was positive for influenza virus given a) the low rates of infection in this age-group[5] and b) that the majority of viral shedding in influenza occurs in the first 2-3 days after symptom onset[14] whereas most reported respiratory symptoms in study participants occurred 8-14 days before admission. Based on our findings it seems unlikely that any delayed cardiac effect of influenza is linked to ongoing or prolonged virus replication or shedding in the respiratory tract. 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[15] but its effect with the pandemic strain H1N1pdm09 is unclear. It has previously been noted that serological studies carried out during the 2009 influenza pandemic were severely hampered by cross reactivity both with vaccine and with seasonal influenza strains[7].

Previous observational studies using large electronic primary care databases have found an association between GP consultation for acute respiratory infection in the last month and risk of acute myocardial infarction[16-18]. Although studies were conducted over different time periods, they encompassed the effect of varying seasonal influenza strains. In the present study, we controlled for important potential confounders such as influenza vaccination status, and showed that statin use was equally prevalent in cases and controls. We did not, however, have complete data on other drugs that have been hypothesised to have immune-regulatory effects (such as ACE inhibitors ,angiotensin receptor blockers (ARBs), metformin, glitazones and fibrates). If these agents reduce the likelihood of people experiencing ILI and were more commonly used in cases than controls they could potentially have confounded the relationship between ILI and AMI. It was reassuring, however, that our results were consistent with those obtained in our recent self-controlled case series study - a design that implicitly controls for fixed confounders. In this study we used linked primary care and cardiac disease registry records from 3,927 patients from 2003-2009, which also included acute respiratory infection consultations occurring during the first wave of H1N1pdm09 circulation[19]. We found an incidence ratio for acute myocardial infarction of 4.19 (95% confidence interval 3.18-5.53) in the first 1-3 days after acute respiratory infection, with the risk falling to baseline after 28 days[19]. Elderly people and those consulting for an infection judged most likely to be due to influenza were at greatest risk. During the 2009 influenza pandemic people with underlying cardiovascular disease were much more likely to be hospitalised[13] and to die[20] from a range of causes attributable to pandemic influenza. Although most deaths from H1N1pdm09 occurred in younger people, this was partly a function of the age distribution of infections: in the UK the case fatality rate in the elderly was much higher than in younger age-groups[5] but overall numbers of deaths were small as few elderly people were infected.

Various biological mechanisms are proposed to underlie a relationship between influenza or acute respiratory infection and myocardial infarction[21]. Acute respiratory infections may result in a host of acute inflammatory and haemostatic effects leading to systemic inflammation, altered plasma viscosity, coagulability and haemodynamic changes[22] as well as promoting local endothelial dysfunction, coronary inflammation and plaque rupture[23]. Immobility associated with bed-rest and dehydration might potentiate these processes.

In conclusion, this study suggests that recent ILI occurring during the 2009 influenza pandemic was more common in AMI patients. Taken in the context of previous work, this helps to support the hypothesis that, as with other influenza strains, H1N1pdm09 could potentially trigger AMI in vulnerable groups. It is likely, however, that the effect is not specific to influenza and could have also been due to other viruses circulating at the time. The population impact of H1N1pdm09 on rates of hospitalisations and deaths from myocardial infarction is also likely to have been relatively low given the mismatch between the ages of those typically affected by H1N1pdm09 and acute coronary events as well as the relatively mild clinical effects of this influenza strain.

Acknowledgments

We thank all patients who agreed to participate in this study and nurses on the cardiology and surgical wards at the Royal Free Hospital who helped to identify potential participants. We acknowledge Mauli Patel at the Royal Free Virology Laboratory who kindly processed, stored and tested samples.

Contributors

CWG conceived and designed the study, recruited participants, collected and analysed data and wrote the manuscript. AMG, GH, RDR, LS and ACH assisted with study design. AMG provided laboratory resources. CWG, AMG, RDR, LS and ACH interpreted results. AMG, GH, RDR, LS and ACH critically revised the manuscript for intellectual content. LS and ACH provided supervision. All authors approved the final version of the manuscript.

Funding statement

This work was funded by a Medical Research Council Clinical Research Training Fellowship for CWG, grant number G0800689. LS is supported by a Wellcome Trust Senior Research Fellowship in Clinical Science, grant number 098504/Z/12/Z.

Competing interests statement

None declared

Data sharing statement

No additional data are available

References

1. Cox NJ, Subbarao K (1999) Influenza. The Lancet 354: 1277–1282.
2. Warren-Gash C, Smeeth L, Hayward AC (2009) Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. The Lancet Infectious Diseases 9: 601–610.
3. Swine influenza A (H1N1) infection in two children – Southern California, March-April 2009 (2009). MMWR Morb Mortal Wkly Rep 58: 400–402.
4. Outbreak of swine-origin influenza A (H1N1) virus infection – Mexico, March-April 2009 (2009). MMWR Morb Mortal Wkly Rep 58: 467–470.
5. Health Protection Agency (2010) Epidemiological report of pandemic (H1N1) 2009 in the United Kingdom: April 2009 - May 2010.
6. McLean E, Pebody RG, Campbell C, Chamberland M, Hawkins C, et al. (2010) Pandemic (H1N1) 2009 influenza in the UK: clinical and epidemiological findings from the first few hundred (FF100) cases. Epidemiol Infect 138: 1531–1541.
7. Lagacé-Wiens PRS, Rubinstein E, Gumel A (2010) Influenza epidemiology – past, present, and future. Crit Care Med 38: e1–e9.
8. Chacko B, Peter JV, Pichamuthu K, Ramakrishna K, Moorthy M, et al. (2012) Cardiac manifestations in patients with pandemic (H1N1) 2009 virus infection needing intensive care. J Crit Care 27: 106.e1–6.
9. Martin SS, Hollingsworth CL, Norfolk SG, Wolfe CR, Hollingsworth JW. (2010) Reversible cardiac dysfunction associated with pandemic 2009 influenza A (H1N1). Chest 137: 1195-7.
10. Lee N, Chan PKS, Lui GCY, Wong BCK, Sin WWY, et al. (2011) Complications and Outcomes of Pandemic 2009 Influenza A (H1N1) Virus Infection in Hospitalized Adults: How Do They Differ From Those in Seasonal Influenza? J Infect Dis 203: 1739–1747.
11. Dawood FS, Iuliano AD, Reed C, Meltzer MI, Shay DK, et al. (2012) Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. The Lancet Infectious Diseases 12: 687–695.
12. Khanom AB, Velvin C, Hawrami K, Schutten M, Patel M, et al. (2011) Performance of a nurse-led paediatric point of care service for respiratory syncytial virus testing in secondary care. J Infect 62: 52–58.

13. Campbell CNJ, Mytton OT, McLean EM, Rutter PD, Pebody RG, et al. (2010) Hospitalization in two waves of pandemic influenza A(H1N1) in England. Epidemiology & Infection 139: 1560–1569.
14. Lau LLH, Cowling BJ, Fang VJ, Chan K-H, Lau EHY, et al. (2010) Viral shedding and clinical illness in naturally acquired influenza virus infections. J Infect Dis 201: 1509–1516.
15. Rothbarth PH, Groen J, Bohnen AM, De Groot R, Osterhaus AD (1999) Influenza virus serology – a comparative study. J Virol Methods 78: 163–169.
16. Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, et al. (2004) Risk of myocardial infarction and stroke after acute infection or vaccination. New England Journal of Medicine 351: 2611–2618.
17. Clayton TC, Thompson M, Meade TW (2008) Recent respiratory infection and risk of cardiovascular disease: case-control study through a general practice database. Eur Heart J 29: 96–103.
18. Meier CR, Jick SS, Derby LE, Vasilakis C, Jick H, et al. (1998) Acute respiratory-tract infections and risk of first-time acute myocardial infarction. The Lancet 351: 1467–1471.
19. Warren-Gash C, Hayward AC, Hemingway H, Denaxas S, Thomas SL, et al. (2012) Influenza infection and risk of acute myocardial infarction in England and Wales: a CALIBER self-controlled case series study. J Infect Dis 206: 1652–1659.
20. Mytton OT, Rutter PD, Mak M, Stanton E, Sachedina N, et al. (2012) Mortality due to pandemic (H1N1) 2009 influenza in England: a comparison of the first and second waves. Epidemiology & Infection 140: 1533–1541.
21. Bazaz R, Marriott HM, Francis SE, Dockrell DH (2013) Mechanistic links between acute respiratory tract infections and acute coronary syndromes. J Infect 66: 1-17.
22. Harskamp RE, Van Ginkel MW (2008) Acute respiratory tract infections: a potential trigger for the acute coronary syndrome. Annals of Medicine 40: 121–128.
23. Vallance P, Collier J, Bhagat K (1997) Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? The Lancet 349: 1391–1392.

Figure legends

Figure 1 Number of study participants admitted by influenza surveillance week compared to weekly ILI rates per 100,000 based on national RCGP surveillance data

Figure 2 Percentage of cases (n=70) and controls (n=64) reporting various symptoms during respiratory illness

Characteristic	Category	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)	
Admission month	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)	
BMI category	18.5-24.9	20 (30.8)	23 (39.0)	0.41
	25.0-29.9	36 (55.4)	24 (40.7)	
	30.0-39.9	8 (12.3)	10 (17.0)	
	40.0-max	1 (1.5)	2 (3.4)	
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)	
Past medical history	Hypercholesterolaemia*	34 (48.6)	28 (43.8)	0.58
	Diabetes	14 (20.0)	12 (18.8)	0.86
	Hypertension	37 (52.9)	26 (40.6)	0.16
	AMI	14 (20.0)	5 (7.8)	0.04
	Stroke	1 (1.4)	4 (6.3)	0.14
Family history	AMI	43 (61.4)	19 (29.7)	<0.001
	Stroke	5 (7.1)	6 (9.4)	0.64
Influenza vaccination status†	Vaccinated	30 (42.9)	29 (45.3)	0.78

Table 1 Characteristics of study participants (n=134)

*28 cases (40%) and 25 controls (39%) with hypercholesterolaemia reported current statin use

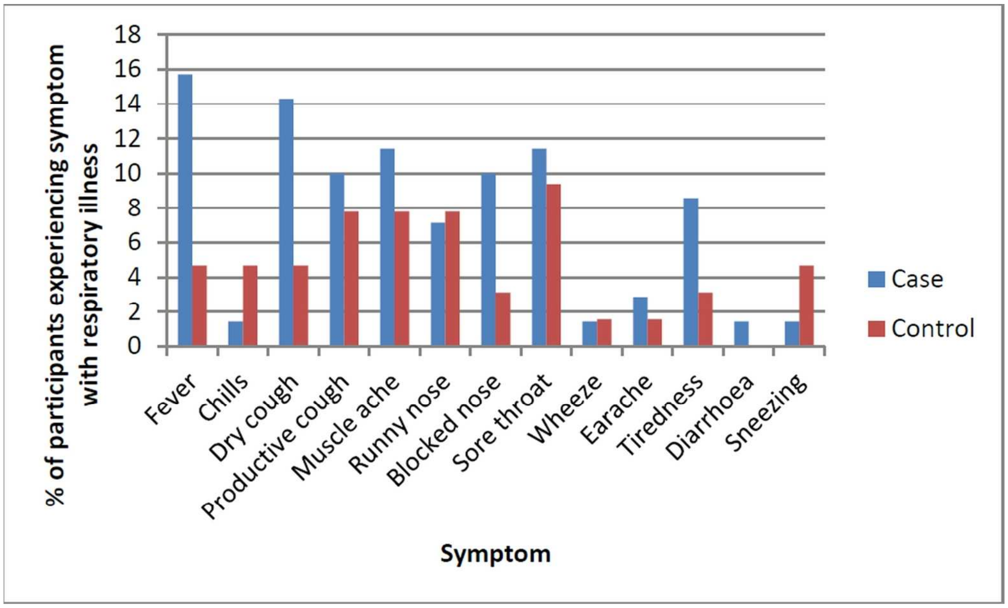
†'Vaccinated' refers to receiving influenza vaccination in the current vaccination year (since September 2009).

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. Muscle ache	8 (11.4)	5 (7.8)	1.52 (0.47-4.92)	2.29 (0.59-8.92)
7. Influenza A IgA antibodies±	25 (46.3)	28 (54.9)	0.71 (0.33-1.53)	0.82 (0.34-2.00)

Table 2 Odds ratios for the association between acute myocardial infarction and various respiratory illness exposure variables, 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 8 equivocal results were excluded, compared to n=134 (70 cases and 64 controls) for all other exposures.



Percentage of cases (n=70) and controls (n=64) reporting various symptoms during respiratory illness
150x90mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Reported on page no.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5
		(*b*) For matched studies, give matching criteria and the number of controls per case	Frequency matching criteria given on p5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	Study size based on numbers of eligible patients hospitalised during the influenza circulation period
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how matching of cases and controls was addressed	N/A

		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	Data not systematically collected but reasons for non-participation include lack of time and feeling unwell during admission
		(c) Consider use of a flow diagram	Not done as no follow up with this study design. Fig 1 shows recruitment over time.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1 and p8
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9-10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.