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Early View

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# Laboratory-confirmed respiratory infections as triggers for acute myocardial infarction and stroke: a self-controlled case series analysis of national linked datasets from Scotland

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**Laboratory-confirmed respiratory infections as triggers for acute myocardial infarction and stroke: a self-controlled case series analysis of national linked datasets from Scotland.**

**Short title**: Lab-confirmed respiratory infections as MI and stroke triggers

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**Take home message** Laboratory-confirmed respiratory infections are linked to strokes and heart attacks in a Scottish population

**Keywords**: *S.pneumoniae*; influenza; respiratory viruses; myocardial infarction; stroke; self-controlled case series

**ABSTRACT**

While acute respiratory infections can trigger cardiovascular events, the differential effect of specific organisms is unknown. This is important to guide vaccine policy.

Using national infection surveillance data linked to the Scottish Morbidity Record, we identified adults with a first myocardial infarction (MI) or stroke from 01/01/2004 to 31/12/2014 and a record of laboratory-confirmed respiratory infection during this period. Using self-controlled case series analysis, we generated age- and season-adjusted incidence ratios (IR) for MI (n=1,227) or stroke (n=762) after infections compared to baseline time.

We found substantially increased MI rates in the week after *S.pneumoniae* and influenza: adjusted IRs for days 1-3 were 5.98, 95% CI 2.47-14.4, and 9.80, 95% CI 2.7-40.5, respectively. Rates of stroke after infection were similarly high and remained elevated to 28 days: day 1-3 adjusted IRs 12.3, 95% CI 5.48-27.7, and 7.82, 95% CI 1.07-56.9, for *S.pneumoniae* and influenza. Although other respiratory viruses were associated with raised point estimates for both outcomes, only the day 4-7 estimate for stroke reached statistical significance.

We showed a marked cardiovascular triggering effect of *S.pneumoniae* and influenza, which highlights the need for adequate pneumococcal and influenza vaccine uptake. Further research is needed into vascular effects of non-influenza respiratory viruses.

**INTRODUCTION**

Ischaemic heart disease, cerebrovascular disease and lower respiratory infections have been the three leading causes of death globally for more than 15 years [1,2]. Acute respiratory infections are associated with a short-term increase in the risk of acute cardiovascular events [3,4]. A systematic review of observational studies showed that cardiovascular complications affected around 18% of patients hospitalised for community-acquired pneumonia, with 5% experiencing acute coronary syndrome [5]. As the population ages and the prevalence of multi-morbidity increases, understanding and preventing interactions between diseases will become increasingly important.

In population-based studies using primary care electronic health records (EHRs), acute respiratory infections are typically defined by clinical or syndromic criteria, as microbiological testing is rare. In hospital settings, laboratory testing for respiratory organisms is comparatively more common, but is still likely to be limited for patients with a cardiovascular, rather than respiratory, presentation. Despite extensive use of diagnostic tests, no causative organism is identified in around 40% of hospitalised patients with community-acquired pneumonia [6,7]. Inability to discriminate between different causative organisms therefore limits the utility of many existing studies for informing vaccine policy.

An alternative approach is to investigate whether existing vaccines against the two most common preventable respiratory organisms affecting older people – influenza and *Streptococcus pneumoniae* – reduce vascular risk. Meta-analysis of data from randomised controlled trials (RCTs) of influenza vaccine versus placebo in people with existing cardiovascular disease shows a 36% reduction in the risk of major adverse cardiovascular events (RR 0.64, 95% CI 0.48-0.86) associated with influenza vaccine [8]. For pneumococcal vaccine, data on cardiovascular outcomes from RCTs are lacking and selection biases associated with observational vaccine studies are well-described [9]. Nevertheless, a recent systematic review and meta-analyses of case control and cohort data suggested that receipt

of pneumococcal vaccine was associated with a modest cardioprotective effect – pooled RR 0.83 (95% CI 0.71-0.97) from a random effects model [10], which was consistent with the pooled estimate from a similar systematic review [11].

In humans, both influenza and *S.pneumoniae* can have direct myocardial effects [12,13] in addition to non-specific pro-inflammatory and pro-coagulant changes, which are also seen in community-acquired pneumonia and viral upper respiratory tract infections and may exacerbate underlying atherosclerotic disease [14]. Understanding the relative contributions of different respiratory organisms to cardiovascular hospital admissions will inform health service planning and guide the development and targeting of interventions to reduce infection-related vascular risk.

Here we aimed to quantify the association between laboratory-confirmed respiratory bacteria or virus infections and risk of first myocardial infarction (MI) or stroke using self-controlled case series analysis of anonymised linked EHRs from Scotland. This study design is particularly suited to the investigation of transient exposures and acute outcomes, and has the major advantage of controlling implicitly for fixed between-person confounding [15], which may be otherwise difficult to account for using routinely collected health data.

## METHODS

### Data sources

We used general acute hospital inpatient data from the Scottish Morbidity Record (SMR01). This is a large national dataset containing details of all episodes of admitted patient care including daycases from acute specialties from hospitals in Scotland. Data are available from 1968 onwards with more than 1.4 million records added each year. SMR01 records include sociodemographic details, clinical data on diagnoses and procedures undergone as well as administrative information such as admission and discharge dates.

We also obtained data on a range of laboratory-confirmed respiratory organisms from the Electronic Communication of Surveillance in Scotland (ECOSS) dataset. ECOSS comprises information on all identifications of organisms or infections of clinical and public health significance reported from NHS laboratories throughout Scotland. Samples tested for respiratory organisms identified in ECOSS could originate from patients seeking medical attention for respiratory symptoms in either primary or secondary care settings. Extracts from these datasets were linked by the indexing team at National Services Scotland using a deterministic approach based on the CHI number – a unique patient identifier.

**Study design**

We conducted a self-controlled case series study to investigate the relative incidence of MI or stroke occurring in time-periods following laboratory-confirmed respiratory infections compared to baseline time-periods for each individual. Derived from cohort logic, self-controlled case series is a design in which individuals act as their own controls during different time periods. This has the major advantage of eliminating time-invariant confounding [15,16], which is particularly important for analyses involving routine health datasets in which potential confounding factors may not be adequately recorded. Self-controlled case series analysis also allows for the inclusion of time-varying confounders such as age and season in the models. Our null hypothesis was that exposure to acute respiratory organisms would not affect MI and stroke incidence.

Self-controlled case series designs are statistically efficient relative to the cohort method (16). Our sample size calculation, based on a relative incidence of 3, a post-exposure risk period of 28 days and 10 year median follow up duration, showed that 654 cases would be needed to estimate results with 90% power at the 5% significance level.

**Exposures and outcomes**

Records of all hospital admissions for first ICD-10 coded acute MI (codes I21 and I23) and stroke (codes I60, I61 and I63) occurring in individuals aged 40 years or over during the study period of 1 Jan 2004 to 31 December 2014 were obtained from the SMR01 dataset. We included a look-back period of ten years to ensure that individuals did not have an MI or stroke diagnosis before the study period. All linked ECOSS records were then extracted for specimens testing positive for one of the following respiratory organisms: influenza, parainfluenza, rhinovirus, respiratory syncytial virus, human metapneumovirus or *S. pneumoniae* during the same study period. Supplementary table 1 lists the specimen types and testing methods used for different respiratory organisms. For inclusion, a sample had to test positive by any one of the methods listed.

For each person, records of multiple respiratory organisms dated within 28 days of each other were de-duplicated within strata of virus or bacteria. For example, two virus records within 28 days were considered to represent a single episode of infection, with the onset date and diagnosis taken from the earliest record. For inclusion in our analysis, each individual required a record of first MI or stroke and a record of at least one laboratory-confirmed respiratory infection (defined in supplementary table 1) during the study period. We restricted analyses to participants' first MI or stroke, because experiencing a cardiovascular event may alter the risk of subsequent events, and self-controlled case series is based upon the assumption that recurrent events occurring within individuals are independent.

**Statistical analysis**

We used conditional Poisson regression to calculate the relative incidence of first MI and stroke occurring in pre-defined exposure periods after confirmed respiratory infections compared to

unexposed or baseline time periods. We divided the exposure period into days 1-3, 4-7, 8-14 and 15-28 days after acute respiratory infection – see figure 1.

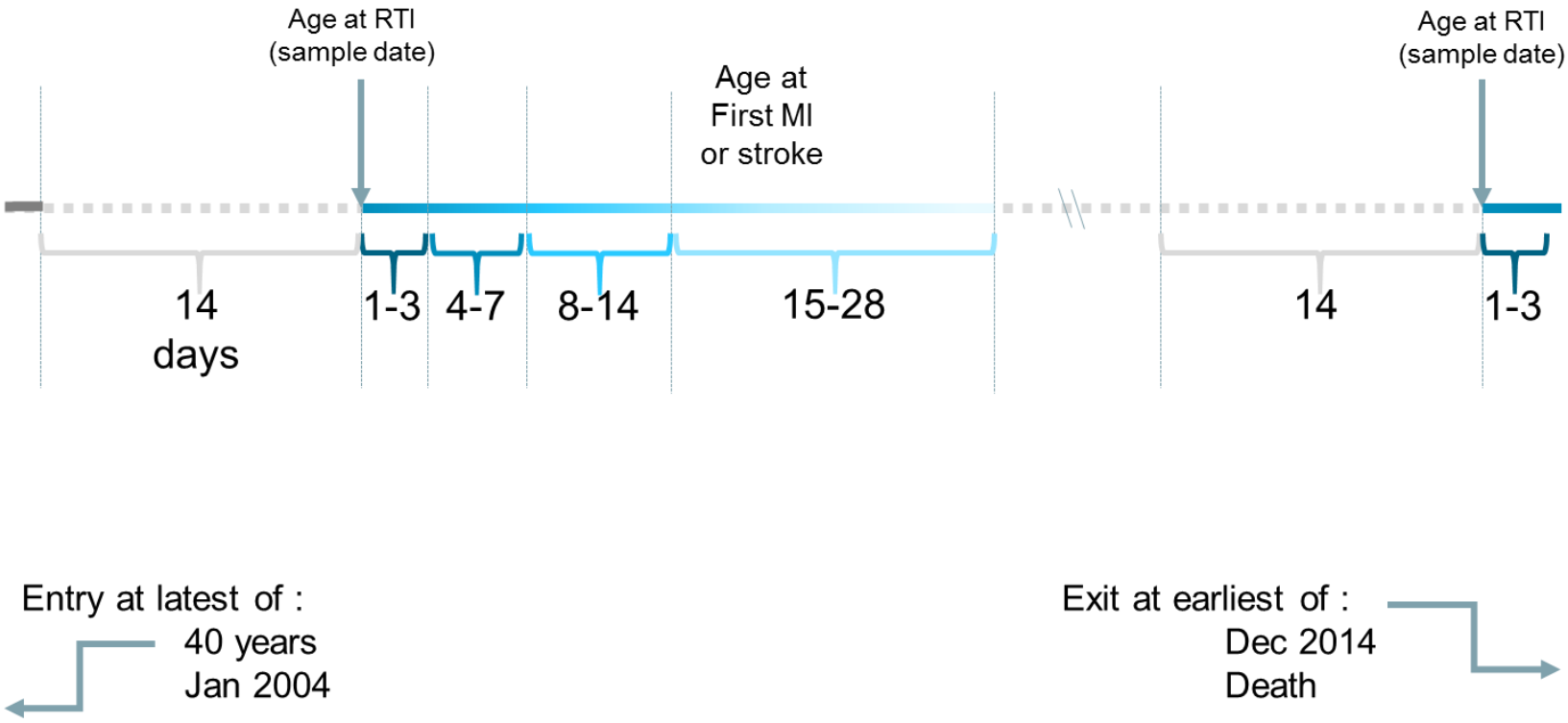


**Figure 1 Self-controlled case series timeline.**

This timeline is based on age and shows the risk period divided into 1-3, 4-7, 8-14 and 25-28 days after respiratory tract infection (RTI). The incidence ratio for cardiovascular events occurring within each risk period compared to baseline time was calculated for each individual.

We defined a separate pre-exposure period of day 0 to day 14 before isolation of a respiratory organism and excluded this from baseline time: having a cardiovascular event in this period (e.g. a stroke) may affect subsequent likelihood of recording of respiratory organisms (e.g. an episode of hospital-acquired pneumonia after stroke). We calculated the incidence ratio for MI or stroke occurring in each exposure period compared with baseline time using conditional Poisson regression models which also adjusted for the time-varying confounders age in 5-year age groups and season in 3-month blocks.

We presented results separately for MI and stroke and for respiratory bacteria (*S. pneumoniae*) and respiratory viruses (combined). In self-controlled case series analysis, the outcome should not censor follow-up so we conducted a sensitivity analysis to assess the effect of excluding records for individuals who died within 30 days of MI or stroke [15]. We also investigated the effect of stratifying by age at cardiovascular event (age<65 years compared to 65 years or over) and by whether respiratory viruses were vaccine-preventable or not (influenza compared to other respiratory viruses). Data were analysed using Stata version 14.0 (Statacorp, TX, USA).

**Ethics statement**

The study was approved by the Public Benefit and Privacy Panel for Health and Social Care, NHS Scotland (Ref: 1516-0263) and the UCL Research Ethics Committee (Project ID: 3898/001).

## RESULTS

### Description of study participants

We included 1,227 individuals with first MI (61% male) and 762 with first stroke (51% male) with a median age of 68 years (IQR 59-77) at the date of cardiovascular event. Patients with MI had records for 924 separate episodes of *S.pneumoniae* infection and 491 episodes of respiratory virus infections during the study period (mean 1.15 samples per person, standard deviation (sd) = 0.55). For stroke patients, the equivalent figures were 576 episodes of *S.pneumoniae* infections and 276 of respiratory viruses respectively (mean 1.12 samples per person, sd = 0.45) (supplementary table 2). Table 1 describes the baseline characteristics of participants.

**Table 1 Baseline characteristics of participants**

| Characteristic | | MI (n=1,227 patients with 1,415 records of respiratory organisms) | | Stroke (n=762 patients with 852 records of respiratory organisms) | |
|---|---|---|---|---|---|
| | | n | % | N | % |
| **Records of respiratory organism*** | *S. pneumoniae* | 924 | 65..3 | 576 | 67..6 |
| | Influenza | 179 | 12..7 | 114 | 13..4 |
| | Rhinovirus | 122 | 8..6 | 79 | 9..3 |
| | Parainfluenza | 69 | 4..9 | 34 | 4..0 |
| | RSV | 69 | 4..9 | 24 | 2..8 |
| | HMPV | 52 | 3..7 | 25 | 2..9 |
| **Age at first cardiovascular (CVD) event (years)** | 40-49 | 120 | 9..8 | 69 | 9..1 |
| | 50-59 | 219 | 17..8 | 136 | 17..8 |
| | 60-69 | 347 | 28..3 | 188 | 24..7 |
| | 70-79 | 363 | 29..6 | 220 | 28..9 |
| | 80-89 | 161 | 13..1 | 131 | 17..2 |
| | 90+ | 17 | 1..4 | 18 | 2..4 |
| **Gender** | Male | 751 | 61..2 | 392 | 51..4 |
| | Female | 476 | 38..8 | 370 | 48..6 |
| **Length of stay associated with CVD event (days)** | Median | 5 | | 11 | |
| | Lower quartile | 3 | | 4 | |
| | Upper quartile | 10 | | 41 | |
| **Died <30 days after CVD event** | Yes | 101 | 8..2 | 107 | 14..0 |
| | No | 1,126 | 91..8 | 655 | 86..0 |
| **Died within study period** | Yes | 473 | 38..5 | 345 | 45..3 |
| | No | 754 | 61..5 | 417 | 54..7 |

* For *S.pneumoniae*, the number of episodes recorded per person across the whole study period for both MI and stroke patients ranged from 1 to 8. For respiratory viruses, the respective numbers of episodes recorded per person ranged from 1 to 3 (influenza), 1 to 3 (rhinovirus), 1 to 4 (parainfluenza), 1 to 2 (RSV) and 1 (HMPV).

**Effect of respiratory organisms on first MI and stroke**

The age- and season-adjusted incidence ratio (IR) for first MI was markedly raised in the first 1-3 days after both respiratory bacterial and viral infections: IR for *S.pneumoniae* 5.98, 95% CI 2.47-14.4, p<0.001; IR for viruses 5.59, 95% CI 1.77-17.6, p=0.003, and persisted for around one week. For first episodes of stroke, the point estimate was even higher: adjusted IR for *S.pneumoniae* 12.3, 95% CI 5.48-27.7, p<0.001; adjusted IR for viruses 6.79, 95% CI 1.67-27.50, p=0.007 for days 1-3. Elevated rates of stroke following both bacterial and viral infections persisted to 28 days (p<0.001). Full results are shown in table 2.

**Table 2 Age- and season-adjusted incidence ratio (IR) for a) first MI and b) first stroke in periods after *S.pneumoniae* and respiratory viruses (combined) compared to baseline time**

| **Outcome: MI, n=1,227** | | | | |
|---|---|---|---|---|
| **Time period (days after sample)** | **IR for *S.pneumoniae* (95% CI)** | **p value** | **IR for respiratory viruses (95% CI)** | **p value** |
| 1-3 | 5.98 (2.47-14.4) | <0.001 | 5.59 (1.77-17.6) | 0.003 |
| 4-7 | 3.79 (1.41-10.1) | 0.008 | 3.00 (0.74-12.1) | 0.12 |
| 8-14 | 1.65 (0.53-5.15) | 0.38 | 1.00 (0.14-7.15) | 0.99 |
| 15-28 | 2.04 (0.96-4.31) | 0.06 | 2.12 (0.79-5.70) | 0.13 |
| Baseline | 1.00 | - | 1.00 | - |
| **Outcome: stroke, n=762** | | | | |
| **Time period (days after sample)** | **IR for *S.pneumoniae* (95% CI)** | **p value** | **IR for respiratory viruses (95% CI)** | **p value** |
| 1-3 | 12.30 (5.48-27.70) | <0.001 | 6.79 (1.67-27.50) | 0.007 |
| 4-7 | 8.23 (3.39-19.90) | <0.001 | 5.43 (1.34-21.90) | <0.001 |
| 8-14 | 4.90 (2.02-11.80) | <0.001 | 5.01 (1.59-15.70) | <0.001 |
| 15-28 | 4.09 (2.02-8.27) | <0.001 | 4.02 (1.62-9.95) | <0.001 |
| Baseline | 1.00 | - | 1.00 | - |

**Sensitivity analysis removing fatal events**

Excluding 101 MI records with a date of death within 30 days (8% of the total) made little difference to the overall findings: in this analysis, the age and season-adjusted IR for MI associated with *S.pneumoniae* was 5.41, 95% CI 2.02-14.5) in days 1-3; for respiratory viruses, the equivalent adjusted IR was 6.20, 95% CI 1.97-19.6. Patterns across the 1 to 28 day risk period were similar to the main model. For stroke, 107 records of fatal events (14% of the total) were excluded. After this, the new adjusted IR associated with *S.pneumoniae* was 9.87, 95% CI 3.67-26.5 in days 1-3, and all incidence ratios remained elevated to 28 days. In contrast, for respiratory viruses, although all point estimates were elevated, only the day 8-14 and 15-28 day estimates reached statistical significance. Table 3 shows the full results of this sensitivity analysis.

**Table 3 Age- and season-adjusted incidence ratio (IR) for a) first MI with ≥30 day survival and b) first stroke with ≥30 day survival in periods after *S.pneumoniae* and respiratory viruses (combined) compared to baseline time**

| Outcome: non-fatal MI, n=1,126 | | | | |
|---|---|---|---|---|
| **Time period (days after sample)** | **IR for *S.pneumoniae* (95% CI)** | **p value** | **IR for respiratory viruses (95% CI)** | **p value** |
| 1-3 | 5.41 (2.02-14.5) | 0.001 | 6.20 (1.97-19.6) | 0.002 |
| 4-7 | 3.13 (1.01-9.75) | 0.049 | 3.25 (0.80-13.1) | 0.10 |
| 8-14 | 1.78 (0.57-5.56) | 0.32 | 1.06 (0.15-7.53) | 0.96 |
| 15-28 | 2.19 (1.04-4.62) | 0.04 | 2.23 (0.83-5.98) | 0.11 |
| Baseline | 1.00 | - | 1.00 | - |
| **Outcome: non-fatal stroke, n=655** | | | | |
| **Time period (days after sample)** | **IR for *S.pneumoniae* (95% CI)** | **p value** | **IR for respiratory viruses (95% CI)** | **p value** |
| 1-3 | 9.87 (3.67-26.5) | <0.001 | 3.97 (0.55-28.5) | 0.17 |
| 4-7 | 7.60 (2.83-20.4) | <0.001 | 3.15 (0.44-22.6) | 0.25 |
| 8-14 | 5.34 (2.20-13.0) | <0.001 | 5.74 (1.82-18.1) | 0.003 |
| 15-28 | 3.90 (1.84-8.27) | <0.001 | 3.70 (1.35-10.1) | 0.01 |
| Baseline | 1.00 | - | 1.00 | - |

**Stratified analysis**

**i) By organism (influenza versus all other respiratory viruses)**

In general, influenza was associated with higher incidence ratios for both MI and stroke than all other respiratory viruses combined, although numbers in some cells were small and confidence intervals overlapped. For MI, the adjusted day 1-3 IR for influenza was 9.80, 95% CI 2.37-40.5, and for other respiratory viruses the IR was 2.81, 95% CI 0.39-20.3. For stroke, the day 1-3 IR after influenza was 7.82, 95% CI 1.07-56.9, and for other respiratory viruses it was 4.86, 95% CI 0.67-35.4. IRs for other time periods are shown in table 4.

**Table 4 Age- and season-adjusted incidence ratio (IR) for a) first MI and b) first stroke after influenza) and all other respiratory viruses (combined) compared to baseline time periods**

| Outcome: MI n=1,126 | | | | |
|---|---|---|---|---|
| **Time period (days after sample)** | **IR for influenza (95% CI)** | **p value** | **IR for other respiratory viruses (95% CI)** | **p value** |
| 1-3 | 9.80 (2.37-40.5) | 0.002 | 2.81 (0.39-20.3) | 0.31 |
| 4-7 | 3.98 (0.55-28.9) | 0.17 | 2.30 (0.32-16.6) | 0.41 |
| 8-14 | 2.72 (0.38-19.5) | 0.32 | ~0 | 0.98 |
| 15-28 | 2.77 (0.68-11.2) | 0.15 | 1.58 (0.39-6.41) | 0.64 |
| Baseline | 1.00 | - | 1.00 | - |
| **Outcome: stroke n=762** | | | | |
| **Time period (days after sample)** | **IR for influenza (95% CI)** | **p value** | **IR for other respiratory viruses (95% CI)** | **p value** |
| 1-3 | 7.82 (1.07-56.9) | 0.042 | 4.86 (0.67-35.4) | 0.12 |
| 4-7 | ~0 | 0.99 | 8.72 (2.14-35.6) | 0.003 |
| 8-14 | 8.13 (1.98-33.3) | 0.004 | 2.35 (0.32-17.0) | 0.40 |
| 15-28 | 5.13 (1.55-17.0) | 0.007 | 2.59 (0.63-10.6) | 0.19 |
| Baseline | 1.00 | - | 1.00 | - |

### ii) By age

Stratifying by age at cardiovascular event was also hampered by small numbers and some cells contained zero events. Nevertheless, those aged less than 65 years tended to have higher adjusted IRs for MI and stroke after respiratory bacteria and virus infections than people aged 65 years or over, although there were overlapping confidence intervals. Full results are shown in table 5.

**Table 5 Age- and season-adjusted incidence ratio (IR) for a) first MI and b) first stroke stratified by age under/≥ 65 years in periods after *S.pneumoniae* and respiratory viruses (combined) compared to baseline time**

| **Outcome: MI aged under 65, n=498** | | | | |
|---|---|---|---|---|
| **Time period (days after sample)** | **IR for *S.pneumoniae* (95% CI)** | **p value** | **IR for respiratory viruses (95% CI)** | **p value** |
| 1-3 | 7.97 (1.97-32.2) | 0.004 | 16.1 (5.12-50.9) | <0.001 |
| 4-7 | 12.7 (4.69-34.4) | <0.001 | 4.20 (0.59-30.1) | 0.15 |
| 8-14 | 1.77 (0.25-12.7) | 0.572 | ~0 | 0.997 |
| 15-28 | 1.93 (0.48-7.81) | 0.355 | 4.08 (1.29-12.9) | 0.017 |
| Baseline | 1.00 | - | 1.00 | - |
| **Outcome: MI aged 65 years and over, n=729** | | | | |
| **Time period (days after sample)** | **IR for *S.pneumoniae* (95% CI)** | **p value** | **IR for respiratory viruses (95% CI)** | **p value** |
| 1-3 | 5.16 (1.65-16.1) | 0.005 | ~0 | 0.998 |
| 4-7 | ~0 | 0.998 | 2.35 (0.33-17.0) | 0.40 |
| 8-14 | 1.60 (0.40-6.45) | 0.51 | 1.57 (0.22-11.2) | 0.65 |
| 15-28 | 2.09 (0.86-5.06) | 0.10 | 0.82 (0.11-5.9) | 0.85 |
| Baseline | 1.00 | - | 1.00 | - |
| **Outcome: stroke aged under 65 years, n=301** | | | | |
| **Time period (days after sample)** | **IR for *S.pneumoniae* (95% CI)** | **p value** | **IR for respiratory viruses (95% CI)** | **p value** |
| 1-3 | 37.4 (13.7-102) | <0.001 | 23.4 (5.71-96.3) | <0.001 |
| 4-7 | 7.83 (1.09-56.4) | 0.041 | ~0 | 0.998 |
| 8-14 | 14.2 (4.48-45.2) | <0.001 | 10.5 (2.55-42.9) | 0.001 |
| 15-28 | 9.74 (3.56-26.7) | <0.001 | 5.90 (1.68-20.7) | 0.006 |
| Baseline | 1.00 | - | 1.00 | - |
| **Outcome: stroke aged 65 years and over, n=461** | | | | |
| **Time period (days after sample)** | **IR for *S.pneumoniae* (95% CI)** | **p value** | **IR for respiratory viruses (95% CI)** | **p value** |
| 1-3 | 5.21 (1.29-21.0) | 0.02 | ~0 | 0.999 |
| 4-7 | 7.69 (2.84-20.8) | <0.001 | 6.83 (1.66-28.1) | 0.008 |
| 8-14 | 2.35 (0.58-9.48) | 0.23 | 2.33 (0.32-16.8) | 0.40 |
| 15-28 | 2.45 (0.91-6.60) | 0.08 | 2.52 (0.62-10.3) | 0.20 |
| Baseline | 1.00 | - | 1.00 | - |

**DISCUSSION**

We show that infection with *S. pneumoniae* and a range of different respiratory viruses is associated with increased rates of first MI and stroke in a Scottish adult population. The period of elevated risk associated with infections persisted to 28 days for stroke but was more transient for MI, suggesting potentially different mechanisms. While the vaccine-preventable organisms influenza and *S.pneumoniae* were associated with particularly high incidence ratios for vascular events, we also showed an effect of other viruses, for which vaccines are not currently available, although this was significant only for stroke. The finding of higher incidence ratios in people aged under 65 years warrants further exploration but may relate to vaccine: influenza vaccine uptake reached 77% in Scotland during the period of observation among those aged ≥65 years [17] but was much lower in younger people, who may not be eligible for vaccination. This highlights the need to consider other prevention and treatment options for acute respiratory infections, especially among patients at high risk of cardiovascular disease.

Strengths of our study include the novel use of data on laboratory-confirmed respiratory infections from eleven years of national surveillance in Scotland linked to robust vascular outcome measures from national hospitalisation records. A unique advantage of the self-controlled case series method is its ability to control for fixed confounders [15], which may distort findings from other similar studies using routine health data. We also controlled for the time-varying effects of age and season, so residual confounding by other time-varying factors is unlikely. We showed a biological gradient of stroke and, to a lesser extent, MI risk after acute respiratory infections, which is likely to indicate a causal relationship. Use of laboratory-confirmed infection measures improved diagnostic specificity and therefore the relevance of results for vaccine policy. Our population-based approach means that results should be generalizable to similar Northern European populations.

Our study nevertheless had some limitations. Although primary analyses were well-powered and self-controlled case series is an efficient design, the relatively small population size in Scotland meant that the study lacked power for some sub-group analyses. It was not possible to investigate the effects of individual non-influenza respiratory viruses on vascular events or to examine age effects in detail. We were also unable to investigate the effect of co-infections separately, although the number of patients experiencing co-infections was extremely small. During the study period there were changes in microbiological testing practices e.g. PCR was not widely used in 2004. However, if testing methods were relatively less sensitive in the earlier years of our study, this would potentially reduce the number of patients meeting inclusion criteria (and therefore statistical power) rather than introduce bias. While we could not exclude the chance that some organisms isolated might represent incidental colonisation rather than symptomatic infections, this would have the effect of biasing results towards the null. In addition, we used the day after the date of respiratory sampling as the start of the risk period. However, the infection onset date would have occurred earlier, most likely during the 14-day period excluded from baseline time, which would have led to underestimation of the true effect size.

The effect of influenza virus on cardiovascular hospital admissions and deaths is well-documented in different settings [18,19]. Recent studies describe high rates of morbidity and seasonal mortality among older adults infected with other respiratory viruses including human metapneumovirus, parainfluenza, rhinovirus and respiratory syncytial virus [20–23]. We recently showed that circulation of adenovirus, human metapneumovirus, influenza, rhinovirus and respiratory syncytial virus was associated with hospitalisations for cardiovascular disease at population level [24]. In this self-controlled case series analysis, we confirm and extend findings from individual-level studies using primary care data that show an acute triggering effect of clinically-diagnosed acute respiratory infections on MI and stroke [25,26]. The higher point estimates for influenza compared to other

respiratory viruses are consistent with previous findings suggesting that cardiovascular complications are more frequent with influenza than other viruses such as rhinovirus among hospitalised adults [20].

Our findings that both influenza and *S.pneumoniae* have specific triggering effects on MI and stroke emphasise the need to encourage uptake of influenza and pneumococcal vaccines wherever indicated, especially among populations with existing heart disease in whom influenza vaccine uptake is sub-optimal [27]. Trials are ongoing to investigate the effectiveness of pneumococcal vaccine against cardiovascular events [28], and the effects of different formulations and target populations for influenza vaccination on individual cardiovascular endpoints (NCT02831608, NCT02762851, NCT02787044, NCT02268500 from clinicaltrials.gov). Demonstration of significant cardiovascular protection will inform updated cost effectiveness analyses, and consideration of extending indication for these vaccines to at-risk groups who do not meet current guidelines for vaccination.

The finding that other respiratory viruses for which vaccines are not available also act as cardiovascular triggers merits further exploration. It potentially strengthens the case for considering anti-thrombotic strategies during acute respiratory infections for vulnerable groups, especially as recent evidence points to a dual effect of acute respiratory infections and symptomatic non-steroidal anti-inflammatory drug treatment on cardiovascular risk [29]. Future research should focus on informing development and delivery of stratified interventions to reduce vascular risk associated with a range of respiratory organisms. Large robust population studies are needed to investigate the effects of acute respiratory infections on vascular risk in different population sub-groups e.g. those with individual cardiovascular risk factors and for sub-sets of infections such as bacteria causing atypical pneumonias and, in particular, non-influenza respiratory viruses.

In conclusion, we show a marked cardiovascular triggering effect of *S.pneumoniae* and influenza, as well as raised point estimates for MI and stroke associated with other confirmed respiratory viruses.

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**Contributors**

All authors contributed to design of the study and/or analyses, interpretation of data and critical revision of the manuscript for important intellectual content. RB and HW performed statistical analyses. CWG obtained funding and drafted the paper. All authors approved the final manuscript version.

**Declaration of interests**

All authors declare no conflicts of interest.

**REFERENCES**

1. Wang H, Naghavi M, Allen C, et al. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet* 2016; 388(10053): 1459–544.
2. World Health Organization. The top 10 causes of death. Jan 2017. Accessed 20/07/2017. Available from: http://www.who.int/mediacentre/factsheets/fs310/en/index.html
3. Barnes M, Heywood AE, Mahimbo A, et al. Acute myocardial infarction and influenza: a meta-analysis of case-control studies. *Heart* 2015; 101(21): 1738–47.
4. Warren-Gash C, Smeeth L, Hayward AC. Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. *Lancet Infect Dis* 2009; 9(10): 601–10.
5. Corrales-Medina VF, Suh KN, Rose G, et al. Cardiac Complications in Patients with Community-Acquired Pneumonia: A Systematic Review and Meta-Analysis of Observational Studies. Klugman KP, editor. *PLoS Med* 2011; 8(6): e1001048.
6. Huijskens EGW, van Erkel AJM, Palmen FMH, et al. Viral and bacterial aetiology of community-acquired pneumonia in adults. *Influenza Other Respir Viruses* 2013; 7(4): 567–73.

7. Hirama T. A Real-Time PCR-Based Diagnostic Test for Organisms in Respiratory Tract Infection. 2016. Accessed 23 May 2017. Available from: http://www.intechopen.com/books/polymerase-chain-reaction-for-biomedical-applications/a-real-time-pcr-based-diagnostic-test-for-organisms-in-respiratory-tract-infection

8. Udell JA, Zawi R, Bhatt DL, et al. Association Between Influenza Vaccination and Cardiovascular Outcomes in High-Risk Patients: A Meta-analysis. *JAMA* 2013; 310(16): 1711.

9. Simonsen L, Taylor RJ, Viboud C, Miller MA, Jackson LA. Mortality benefits of influenza vaccination in elderly people: an ongoing controversy. *Lancet Infect Dis* 2007; 7(10): 658–66.

10. Ren S, Newby D, Li SC, et al. Effect of the adult pneumococcal polysaccharide vaccine on cardiovascular disease: a systematic review and meta-analysis. *Open Heart* 2015; 2(1): e000247.

11. Vlachopoulos CV, Terentes-Printzios DG, Aznaouridis KA, et al. Association between pneumococcal vaccination and cardiovascular outcomes: a systematic review and meta-analysis of cohort studies. *Eur J Prev Cardiol* 2015; 22(9): 1185–99.

12. Ison MG, Campbell V, Rembold C, et al. Cardiac Findings during Uncomplicated Acute Influenza in Ambulatory Adults. *Clin Infect Dis* 2005; 40(3): 415–22.

13. Reyes LF, Restrepo MI, Hinojosa CA, et al. Severe Pneumococcal Pneumonia Causes Acute Cardiac Toxicity and Subsequent Cardiac Remodeling. *Am J Respir Crit Care Med*. 2017; 196(5):609–20.

14. Singanayagam A, Singanayagam A, Elder DHJ, et al. Is community-acquired pneumonia an independent risk factor for cardiovascular disease? *Eur Respir J* 2012; 39(1): 187–96.

15. Petersen I, Douglas I, Whitaker H. Self controlled case series methods: an alternative to standard epidemiological study designs. *BMJ*. 2016; 354:i4515.

16. Whitaker HJ, Farrington CP, Spiessens B, et al. Tutorial in biostatistics: the self-controlled case series method. *Stat Med* 2006; 25(10): 1768–1797.

17. Public Health England. Surveillance of influenza and other respiratory viruses in the United Kingdom: winter 2014 to 2015. Report No.: 2015046. Accessed 20/07/2017. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/429617/Annualreport_March2015_ver4.pdf

18. Yang L, Ma S, Chen PY, et al. Influenza associated mortality in the subtropics and tropics: Results from three Asian cities. *Vaccine* 2011; 29(48): 8909–14.

19. Green HK, Andrews N, Fleming D, et al. Mortality attributable to influenza in England and Wales prior to, during and after the 2009 pandemic. *PloS One* 2013; 8(12): e79360.

20. Hung IFN, Zhang AJ, To KKW, et al. Unexpectedly Higher Morbidity and Mortality of Hospitalized Elderly Patients Associated with Rhinovirus Compared with Influenza Virus Respiratory Tract Infection. *Int J Mol Sci* 2017; 18(2). pii: E259.

21. Widmer K, Zhu Y, Williams JV, et al. Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults. *J Infect Dis* 2012; 206(1): 56–62.

22. van Asten L, van den Wijngaard C, van Pelt W, et al. Mortality attributable to 9 common infections: significant effect of influenza A, respiratory syncytial virus, influenza B, norovirus, and parainfluenza in elderly persons. *J Infect Dis*. 2012;206(5):628–39.

23. Lee N, Lui GCY, Wong KT, et al. High morbidity and mortality in adults hospitalized for respiratory syncytial virus infections. *Clin Infect Dis* 2013; 57(8): 1069–77.

24. Blackburn R, Hayward AC, Pebody R, Warren-Gash C. Abstract no. 44: Respiratory infections as vascular triggers: time-series analysis of hospital admission with myocardial infarction and laboratory-confirmed respiratory infection in England (2004-2015). J Innov Health Inform. 2017;24(1):11.

25. Smeeth L, Thomas SL, Hall AJ, et al. Risk of Myocardial Infarction and Stroke after Acute Infection or Vaccination. *N Engl J Med* 2004; 351(25): 2611–8.

26. Warren-Gash C, Hayward AC, Hemingway H, et al. Influenza Infection and Risk of Acute Myocardial Infarction in England and Wales: A CALIBER Self-Controlled Case Series Study. *J Infect Dis* 2012; 206(11): 1652–9.

27. Public Health England. Influenza immunisation programme for England: GP patient groups data collection survey season 2015-2016. Report No.: 2016087. Accessed 21/06/2017. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/544552/Seasonal_flu_GP_patient_groups_annual_report_2015_2016.pdf

28. Ren S, Hure A, Peel R, et al. Rationale and design of a randomized controlled trial of pneumococcal polysaccharide vaccine for prevention of cardiovascular events: The Australian Study for the Prevention through Immunization of Cardiovascular Events (AUSPICE). *Am Heart J* 2016; 177: 58–65.

29. Wen Y-C, Hsiao F-Y, Chan KA, et al. Acute Respiratory Infection and Use of Nonsteroidal Anti-Inflammatory Drugs on Risk of Acute Myocardial Infarction: A Nationwide Case-Crossover Study. *J Infect Dis* 2017; 215(4): 503–9.

| Organism | Sample type | Testing method |
|---|---|---|
| Human metapneumovirus | Swab, respiratory sample | PCR, IF |
| Influenza viruses | Swab, respiratory sample, tissue<br><br>Blood | PCR, IF, culture<br><br>CF >128 or >4-fold rise in titre. |
| Parainfluenza | Respiratory sample | PCR, IF, culture |
| Respiratory syncytial virus | Swab, respiratory sample<br><br>Blood | PCR, IF, EIA, culture<br><br>CF>128 or > 4-fold rise in titre |
| Rhinovirus | Respiratory sample | PCR, culture |
| *Streptococcus pneumoniae* | Respiratory sample<br>Blood<br>Blood culture<br>Urine | PCR<br>Antibodies<br>Culture<br>Antigen detection |

**Supplementary table 1 Methods of detection for respiratory organisms used in the Electronic Communication of Surveillance in Scotland (ECOSS) dataset**

PCR – polymerase chain reaction; IF – immunofluorescence; CF – complement fixation; EIA – enzyme immunoassay.

| Outcome: MI | | |
|---|---|---|
| **Specimen type** | ***S.pneumoniae*** | **All respiratory viruses** |
| Sputum | 689 | 38 |
| Saliva | ≤10 samples | 72 |
| Nasal/ throat swab | | 325 |
| Lower respiratory tract sample* | 72 | 21 |
| Blood (serology) | 21 | ≤10 samples |
| Blood (culture) | 107 | |
| Other sample** | 29 | 28 |
| Total | 924 | 491 |
| **Outcome: stroke** | | |
| **Specimen type** | ***S.pneumoniae*** | **All respiratory viruses** |
| Sputum | 367 | 18 |
| Saliva | ≤10 samples | 37 |
| Nasal/ throat swab | | 189 |
| Lower respiratory tract sample* | 76 | 14 |
| Blood (serology) | 25 | ≤10 samples |
| Blood (culture) | 76 | |
| Other sample** | 26 | 14 |
| Total | 576 | 276 |

**Supplementary table 2 Diagnosis of respiratory organisms for MI and stroke patients included in the self-controlled case series analysis**

*Lower respiratory samples included bronchial aspirate, bronchial lavage, bronchial sections, bronchiolar lavage, bronchoalveolar lavage, endotracheal aspirate, endotracheal secretions, endotracheal tube tip, lower respiratory tract aspirate, lung swab, lung tissue, tracheal aspirate, tracheal secretions

**Other samples included aspirate site unspecified, central venous line tip, cerebrospinal fluid, chest drain fluid, corneal scrapings, ear pus, eye swab, fluid unspecified, lymph node, mid-stream urine, mouth swab, oropharyngeal aspirate, PEG tube pus, pleural aspirate, pus site unspecified, respiratory secretions, respiratory unspecified, swab site unspecified, tracheostomy swab, urine