PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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	Warren-Gash, Charlotte; Geretti, Anna Maria; Hamilton, George; Rakhit, Roby; Smeeth, Liam; Hayward, Andrew

VERSION 1 - REVIEW

REVIEWER	Seth S. Martin, MD Fellow, Division of Cardiology The Johns Hopkins Hospital Baltimore, Maryland United States
REVIEW RETURNED	01-Feb-2013

THE STUDY	Why were surgical patients chosen as a control? The rationale for this part of the study design is not explicit in the paper. Why surgical rather than non-cardiovascular medical? The text information offered in the STROBE checklist should be reported in the manuscript. This includes information about how the study size was determined and reasons for non-participation.
GENERAL COMMENTS	The following statement should be revised: “134 participants were recruited, comprising 70 cases and 64 controls, for whom acceptance rates were 66% and 67% respectively.” In this statement, "for whom" refers to the cases and controls, who all accepted participation in the study according to the manuscript. It seems that the authors mean to refer to the full population of individuals that were approached for participation. In addition to percentages, the authors should report the actual number of participants that they approached for the study and how many said yes. Regarding myocardial injury and LV systolic dysfunction, the authors cite two papers from 2012 (references 8 & 9). The original description of LV systolic dysfunction in H1N1pdm09 was offered by our group at Duke in 2010 (Martin et al. Chest 2010;137:1195-7). The important point that we make in that paper, which is not mentioned by the authors, is that the LV systolic dysfunction associated with H1N1pdm09 can often be reversible.

REVIEWER	David S. Fedson, MD Retired
REVIEW RETURNED	06-Feb-2013

GENERAL COMMENTS	I would not publish this paper unless the authors are able to correct the omission noted in the limitation of this study (see attached file). My guess is that this will be very difficult for them to do. Warren-Gash and colleagues have published a very useful review of the association between acute respiratory infection (ARI) and acute myocardial infarction (AMI) (reference 2) and a more recent self-controlled case series study of the association between influenza and AMI (ref 19). In this small case-control study, the authors found that AMI cases were more likely than surgical controls to have had an antecedent history of ILI, although the adjusted OR (3.17) had a very broad confidence interval and was not statistically significant. Cases also had a lower rate of pandemic vaccination, although again the results did not reach statistical significance. Thus this study provides modest encouragement for ideas that have been supported by larger studies in the past; namely, that an ARI or ILI may be followed within a week or two by an AMI, and that influenza vaccination might reduce the occurrence of AMI because it prevents influenza. **Specific comments (in no particular order)** Individuals who were born before 1947 had contact with influenza viruses similar to the pH1N1 virus. Thus, the incidence of pH1N1 infection in people over the age of 52 years would be expected to have been considerably lower that it was in more susceptible younger adults. The median age of AMI patients was 63.6 years, and the great majority were over the age of 52 years. None of the results reported by the authors reached statistical significance. This might have been due, as the authors say, to the rather low incidence of influenza in this older age group that was largely already immune. The authors obtained information on influenza vaccination status, but in the text they don't specify which influenza vaccine they are referring to. A footnote to Table 1says it is pH1N1 vaccine in September 2009 or later. This should also be stated in the text. The study covered the period from 21 September 2009 to 28 February 2010. It is likely that during the first few weeks or months, fewer doses of pandemic vaccine were available than in later months. Approximately half of all cases and controls were hospitalized before December 1st. Was the vaccine widely available before December 1st? It would be interesting if the data for admission months shown for cases and controls in Table 1 also included their vaccination status.

	In the text, the authors state that in their multivariable logistic regression analysis, they controlled for "… age-group, gender, month of admission and influenza vaccination status (all models) and other potential confounding factors." In a footnote to Table 2, they say that adjusted odds ratios were obtained after adjusting for age-group, gender, month of admission, influenza vaccination status, family history of myocardial infarction and personal history of myocardial infarction (exposure variables ILI, fever, cough, and sore throat). Surprisingly, myalgia and headache are not mentioned, although they are classically associated with acute influenza. Moreover, muscle ache figured prominently among the symptoms reported by cases (more so than in controls) in Figure 2. Why was "muscle ache" not included in the logistic regression analysis as an exposure variable? Was it excluded on the basis of the backwards stepwise approach? If so, it might be useful to mention which variables were excluded on the basis of the backwards stepwise approach. Also, why was influenza vaccination status retained in the adjusted analysis, even though vaccination rates in cases and controls were similar (Table 1). Also missing from the adjusted analysis are several classical high-risk conditions (chronic cardiovascular disease [not comprehensively captured by a personal history of AMI or stroke], COPD, renal disease, etc.) These conditions are not listed as among the characteristics of the study participants (Table 1). Readers will wonder why they were not considered in the analysis. Several categories in Table 1 could be simplified because each "Yes" group is simply the reciprocal of the "No" group. If the reader needs the exact number for the reciprocal, he or she can do the maths. **Limitations** Statins are known to reduce the risk of hospitalisation for AMI. (Observational studies also show that statins also decrease the risk of hospitalisation and death due to pneumonia and influenza.) Cases of AMI were almost three times more likely to have a personal history of AMI, and for this reason, might have been more likely than surgical controls to have been taking outpatient statins, although surgical controls had a more frequent personal history of stroke, another indication for taking outpatient statins. Thus outpatient statin treatment could have affected the development of AMI. For this reason, statin treatment must be considered as a potential

	confounding variable and must be included in the adjustment strategy. Other agents with potential immunomodulatory activities – ACE inhibitors, angiotensin receptor blockers (ARBs), metformin, glitazones, and fibrates among them - could also be included. This is a very important limitation of the study: the adjustment strategy is incomplete without these variables.

REVIEWER	Dr. J.V. Peter, MD, DNB, FRACP, FJFICM, FCICM, FICCM, Associate Professor, Medical Intensive Care Unit, Christian Medical College Hospital, There are no competing interests or conflicts
REVIEW RETURNED	07-Feb-2013

REPORTING & ETHICS	Information on Institution Review Board and Ethics approval missing
GENERAL COMMENTS	The authors in this case-control study have attempted to show an association between recent "respiratory illness" and "risk of myocardial infarction". Several published studies, that the authors quoted, have suggested that influenza increases the risk of cardiac events. The authors in the present study concluded that "the study was supportive of the hypothesis that recent ILI was more common in patients hospitalized 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." There are some major concerns that limit the validity of the claims set out by the authors. **Major comments** 1. My major concern is that the proposed association between ILI and myocardial infarction as well as the protective effect of the influenza vaccine are non-significant (OR 3.17, 95%CI 0.61 to 16.47 and 0.46, 95%CI 0.19 to 1.12 respectively). This by itself is not a problem except for the fact that the authors conclude that the study was "supportive of the hypothesis that recent ILI was more common in patients hospitalized with acute myocardial infarction than with acute surgical conditions during the second wave of the influenza A H1H1 pandemic and suggestive of a cardio-protective effect of influenza vaccination". The authors themselves state that the differences were not statistically significant. However the conclusions do not accurately reflect the observations of the authors. 2. As the authors have rightly pointed out, the risk of pandemic H1N1 in the age group at risk for myocardial infarction has been demonstrated in other studies to be "lower" than for other influenza strains. Thus to attribute a relationship

	between influenza A H1N1 and myocardial infarction in this study is probably stretched because of two reasons. First, the serological studies suggested almost an equal exposure to H1N1 in both the cases and controls (46% in cases and 54.9% in controls). It is not clear if the remaining patients had "ILI" or "respiratory infection" due to other non-H1N1 strains of influenza or due to other viruses/bacteria/atypical agents. Even in those who were tested positive on serology, the exposure may have occurred during the first wave of the epidemic, rather the second, particularly if the subjects had experienced more than one episode of ILI during the entire pandemic period. Secondly, the "vulnerable group" for AMI was not the vulnerable group for H1N1 infection. Thus the statement, "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" may be problematic. 3. The data on the protective effects of influenza vaccination is not provided in the text, except for an adjusted OR and CI in page 8. How was this analysis done? I may be missing something. The authors report in the abstract, "cases were more likely than controls to report ILI …. and were less likely to have received influenza vaccination". The number of cases were 70 and controls 64 and according to table 1, 42.9% of the cases received vaccine and 45.3% of the controls did (P=0.78). So how would vaccination be protective? In this context it is also surprising that about 31% of patients who had received the influenza vaccine in the control arm were seronegative (had not seroconverted with the vaccine? – is that what is implied in the text?). The reason for this need to be explained /explored.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

1. Why were surgical patients chosen as a control? The rationale for this part of the study design is not explicit in the paper. Why surgical rather than non-cardiovascular medical?

Response – It was considered that patients with acute non vascular surgical admissions would be suitable controls as these conditions were unlikely to be influenced by recent influenza-like illness. We did not choose elective surgical admissions as these may be cancelled in patients with influenza-like illness. While patients with non-cardiovascular medical conditions might have been suitable controls, there was a greater risk that their presenting complaint might either be triggered or affected by influenza-like illness (eg respiratory illnesses, other infections, exacerbations of chronic inflammatory conditions) so identifying eligible controls from this group would have been more complex and more prone to bias. Reasons for the choice of controls have now been given on p5.

2. The text information offered in the STROBE checklist should be reported in the manuscript. This includes information about how the study size was determined and reasons for non-participation.

Response – The additional text information offered in the STROBE checklist has now been included

in the appropriate sections of the manuscript (p5 for information on how study size was determined and p7 for reasons for non-participation).

3. The following statement should be revised: “134 participants were recruited, comprising 70 cases and 64 controls, for whom acceptance rates were 66% and 67% respectively.” In this statement, "for whom" refers to the cases and controls, who all accepted participation in the study according to the manuscript. It seems that the authors mean to refer to the full population of individuals that were approached for participation. In addition to percentages, the authors should report the actual number of participants that they approached for the study and how many said yes.

Response – This statement has now been revised to ‘134 participants were recruited, who comprised 70 cases from 106 approached (acceptance rate 66%) and 64 controls from 95 approached (acceptance rate 67%)’- p7.

4. Regarding myocardial injury and LV systolic dysfunction, the authors cite two papers from 2012 (references 8 & 9). The original description of LV systolic dysfunction in H1N1pdm09 was offered by our group at Duke in 2010 (Martin et al. Chest 2010;137:1195-7). The important point that we make in that paper, which is not mentioned by the authors, is that the LV systolic dysfunction associated with H1N1pdm09 can often be reversible.

Response – We agree that this key reference should be included and have inserted it in place of our original reference 9. We now also mention the potential reversibility of H1N1pdm09-associated left ventricular systolic dysfunction on p4.

Reviewer 2

1. The authors obtained information on influenza vaccination status, but in the text they don’t specify which influenza vaccine they are referring to. A footnote to Table 1says it is pH1N1 vaccine in September 2009 or later. This should also be stated in the text.

Response – We have now included more detailed information on how influenza vaccination status was captured and categorised in the data sources and measurement section on p6.

2. The study covered the period from 21 September 2009 to 28 February 2010. It is likely that during the first few weeks or months, fewer doses of pandemic vaccine were available than in later months. Approximately half of all cases and controls were hospitalized before December 1st. Was the vaccine widely available before December 1st? It would be interesting if the data for admission months shown for cases and controls in Table 1 also included their vaccination status.

Response – In England & Wales seasonal influenza vaccine was available from September 2009 throughout the study period and pandemic vaccination became available in October 2009. The proportion of study participants who reported being vaccinated in the current season (with one or both vaccines) increased from 0% for those recruited in September 2009 to a peak of 72% for participants recruited in January 2010. This information has now been included in the text on p9 (rather than table 1).

3. In the text, the authors state that in their multivariable logistic regression analysis, they controlled for “… age-group, gender, month of admission and influenza vaccination status (all models) and other potential confounding factors.” In a footnote to Table 2, they say that adjusted odds ratios were obtained after adjusting for age-group, gender, month of admission, influenza vaccination status, family history of myocardial infarction and personal history of myocardial infarction (exposure

variables ILI, fever, cough, and sore throat). Surprisingly, myalgia and headache are not mentioned, although they are classically associated with acute influenza. Moreover, muscle ache figured prominently among the symptoms reported by cases (more so than in controls) in Figure 2. Why was "muscle ache" not included in the logistic regression analysis as an exposure variable? Was it excluded on the basis of the backwards stepwise approach? If so, it might be useful to mention which variables were excluded on the basis of the backwards stepwise approach. Also, why was influenza vaccination status retained in the adjusted analysis, even though vaccination rates in cases and controls were similar (Table 1).

Response – We agree that muscle ache can be a prominent influenza symptom and have therefore included it as an extra exposure in the logistic regression analysis– see table 2. Results are consistent with those based on other respiratory illness symptoms. Influenza vaccination was considered an a priori confounder, so even though vaccination rates were similar in cases and controls this factor was retained in models. This has now been clarified in the statistical methods section on p7.

4. Also missing from the adjusted analysis are several classical high-risk conditions (chronic cardiovascular disease [not comprehensively captured by a personal history of AMI or stroke], COPD, renal disease, etc.) These conditions are not listed as among the characteristics of the study participants (Table 1). Readers will wonder why they were not considered in the analysis.

Response – Our hypothesis was that recent influenza-like illness would be more frequently reported by patients with acute myocardial infarction than control patients. For a factor to confound this association it would have to be independently associated with both outcome (AMI) and exposure (influenza-like illness). Although some of the chronic conditions mentioned would be associated with AMI and might potentially be associated with severity of influenza-like illness, they would not be associated with presence or absence of ILI so were not considered as potential confounders. While it is possible that the presence of chronic conditions might affect an association between AMI and ILI through influencing influenza vaccination status, we controlled for this in the analysis.

5. Several categories in Table 1 could be simplified because each "Yes" group is simply the reciprocal of the "No" group. If the reader needs the exact number for the reciprocal, he or she can do the maths.

Response – We have now simplified table 1 as suggested.

6. Statins are known to reduce the risk of hospitalisation for AMI. (Observational studies also show that statins also decrease the risk of hospitalisation and death due to pneumonia and influenza.) Cases of AMI were almost three times more likely to have a personal history of AMI, and for this reason, might have been more likely than surgical controls to have been taking outpatient statins, although surgical controls had a more frequent personal history of stroke, another indication for taking outpatient statins. Thus outpatient statin treatment could have affected the development of AMI. For this reason, statin treatment must be considered as a potential confounding variable and must be included in the adjustment strategy. Other agents with potential immunomodulatory activities – ACE inhibitors, angiotensin receptor blockers (ARBs), metformin, glitazones, and fibrates among them - could also be included. This is a very important limitation of the study: the adjustment strategy is incomplete without these variables.

Response – We collected data on treatment for hypercholesterolaemia (not shown in the original submission), which showed that levels of statin use were similar in cases (40%) and controls (39%). This has now been reported in a footnote to table 1. We did not consider statin use to be an a priori confounder because, while statins may reduce the risk of hospitalisation and death due to influenza, it

is not clear whether they would affect the presence or absence of ILI (and, as stated in the response to point 4, we were interested in presence or absence of ILI rather than severity of ILI which might be reduced by statin use). As statin use was not associated with the outcome AMI it would have been excluded as a potential confounder on the basis of the backwards stepwise logistic regression.

Reviewer 3

1. My major concern is that the proposed association between ILI and myocardial infarction as well as the protective effect of the influenza vaccine are non-significant (OR 3.17, 95%CI 0.61 to 16.47 and 0.46, 95%CI 0.19 to 1.12 respectively). This by itself is not a problem except for the fact that the authors conclude that the study was "supportive of the hypothesis that recent ILI was more common in patients hospitalized with acute myocardial infarction than with acute surgical conditions during the second wave of the influenza A H1H1 pandemic and suggestive of a cardio-protective effect of influenza vaccination". The authors themselves state that the differences were not statistically significant. However the conclusions do not accurately reflect the observations of the authors.

Response – We agree that by themselves the findings cannot be used to conclude that ILI increases the risk of AMI and that vaccine reduces the risk. We do however think that in the context of results from other studies which show the same direction of effect our results are supportive of these hypotheses. We have amended the wording to make it clear that it is when taken in the context of other work that these results are supportive of the hypotheses (conclusions – p11) and that although our point estimates are in the correct direction, findings do not reach statistical significance (abstract conclusions – p2).

2. As the authors have rightly pointed out, the risk of pandemic H1N1 in the age group at risk for myocardial infarction has been demonstrated in other studies to be "lower" than for other influenza strains. Thus to attribute a relationship between influenza A H1N1 and myocardial infarction in this study is probably stretched because of two reasons. First, the serological studies suggested almost an equal exposure to H1N1 in both the cases and controls (46% in cases and 54.9% in controls). It is not clear if the remaining patients had "ILI" or "respiratory infection" due to other non-H1N1 strains of influenza or due to other viruses/bacteria/atypical agents. Even in those who were tested positive on serology, the exposure may have occurred during the first wave of the epidemic, rather the second, particularly if the subjects had experienced more than one episode of ILI during the entire pandemic period. Secondly, the "vulnerable group" for AMI was not the vulnerable group for H1N1 infection. Thus the statement, "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" may be problematic.

Response – We agree that, based on this study alone, the relationship between influenza A H1N1 and acute myocardial infarction remains unclear. We acknowledge the reviewer's point that ILI in patients in this study could have been attributed to other viruses and so have amended wording in the conclusions to reflect this (p11).

3. The data on the protective effects of influenza vaccination is not provided in the text, except for an adjusted OR and CI in page 8. How was this analysis done? I may be missing something. The authors report in the abstract, "cases were more likely than controls to report ILI …. and were less likely to have received influenza vaccination". The number of cases were 70 and controls 64 and according to table 1, 42.9% of the cases received vaccine and 45.3% of the controls did (P=0.78). So how would vaccination be protective? In this context it is also surprising that about 31% of patients who had received the influenza vaccine in the control arm were seronegative (had not seroconverted with the vaccine? – is that what is implied in the text?). The reason for this need to be explained /explored.

Response – Influenza vaccination status was considered as an additional exposure and uni- and multivariable logistic regression models were generated using the same approach as described for influenza-like illness and respiratory illness exposures. This has been clarified in the statistical methods section on p7. Although similar proportions of cases and controls reported receiving influenza vaccination, in multivariable analysis after controlling for age-group, gender, month of admission and personal history of AMI there was a clear trend towards a protective effect of influenza vaccination on AMI. The results section on p9 now gives details of factors included in the multivariable model.

The fact that 31% of participants who were seronegative had received influenza vaccination does not mean that they had failed to seroconvert with vaccine. Rather, the serological test used – an IgA ELISA – was designed to capture recent exposure to influenza antigen: serum IgA levels peak at around 2 weeks after exposure and fall to baseline by 4-6 weeks. That these participants were seronegative on IgA ELISA reflects the fact that they had received their vaccination more than 4-6 weeks ago and had not been re-exposed to influenza virus in the intervening period. This has been clarified in the methods section on p6.

VERSION 2 – REVIEW

REVIEWER	Fedson, David chemin du Lavoir
REVIEW RETURNED	18-Mar-2013

THE STUDY	"Statins are known to reduce the risk of hospitalisation for AMI. (Observational studies also show that statins also decrease the risk of hospitalisation and death due to pneumonia and influenza.) Cases of AMI were almost three times more likely to have a personal history of AMI, and for this reason, might have been more likely than surgical controls to have been taking outpatient statins, although surgical controls had a more frequent personal history of stroke, another indication for taking outpatient statins. Thus outpatient statin treatment could have affected the development of AMI. For this reason, statin treatment must be considered as a potential confounding variable and must be included in the adjustment strategy. Other agents with potential immunomodulatory activities – ACE inhibitors, angiotensin receptor blockers (ARBs), metformin, glitazones, and fibrates among them - could also be included. This is a very important limitation of the study: the adjustment strategy is incomplete without these variables." The study population included 134 hospitalized patients. Surely, the investigators could have gone back and reviewed the medical records of these patients to determine whether there was evidence of outpatient treatment with any of the medications mentioned above. They could also have determined whether any of these agents had been given to patients after hospital admission. If information on these agents in the medical records was incomplete, this might have been sufficient reason not to include them as variables in the adjusted analysis. If so, the investigators could at least have mentioned this as a limitation of their study in their discussion. They chose to completely ignore this important limitation of their study. Numerous observational studies have been published showing the effectiveness of outpatient statins in reducing hospitalisation and mortality in sepsis, pneumonia and (one study) laboratory-confirmed influenza. Influenza scientists have yet to consider treatment with these agents as potential confounders in

	their analyses of influenza vaccination effectiveness. For this reason, estimates of vaccination effectiveness in older individuals who are often treated with these agents are no longer reliable if these potential confounders are not considered.
RESULTS & CONCLUSIONS	It is no longer acceptable for investigators to ignore the possibility that commonly prescribed medications that have immunomodulatory activities should be considered as potential confounding variables in observational studies such as this. In its current form, this article is unsuitable for publication. If the authors were to revise it in light of the comments above, this recommendation could change.
GENERAL COMMENTS	I have recommended that this article not be published.

VERSION 2 – AUTHOR RESPONSE

"Statins are known to reduce the risk of hospitalisation for AMI. (Observational studies also show that statins also decrease the risk of hospitalisation and death due to pneumonia and influenza.) Cases of AMI were almost three times more likely to have a personal history of AMI, and for this reason, might have been more likely than surgical controls to have been taking outpatient statins, although surgical controls had a more frequent personal history of stroke, another indication for taking outpatient statins. Thus outpatient statin treatment could have affected the development of AMI. For this reason, statin treatment must be considered as a potential confounding variable and must be included in the adjustment strategy. Other agents with potential immunomodulatory activities – ACE inhibitors, angiotensin receptor blockers (ARBs), metformin, glitazones, and fibrates among them - could also be included. This is a very important limitation of the study: the adjustment strategy is incomplete without these variables."

The study population included 134 hospitalized patients. Surely, the investigators could have gone back and reviewed the medical records of these patients to determine whether there was evidence of outpatient treatment with any of the medications mentioned above. They could also have determined whether any of these agents had been given to patients after hospital admission. If information on these agents in the medical records was incomplete, this might have been sufficient reason not to include them as variables in the adjusted analysis. If so, the investigators could at least have mentioned this as a limitation of their study in their discussion. They chose to completely ignore this important limitation of their study. Numerous observational studies have been published showing the effectiveness of outpatient statins in reducing hospitalisation and mortality in sepsis, pneumonia and (one study) laboratory-confirmed influenza. Influenza scientists have yet to consider treatment with these agents as potential confounders in their analyses of influenza vaccination effectiveness. For this reason, estimates of vaccination effectiveness in older individuals who are often treated with these agents are no longer reliable if these potential confounders are not considered.

It is no longer acceptable for investigators to ignore the possibility that commonly prescribed medications that have immunomodulatory activities should be considered as potential confounding variables in observational studies such as this.

Response – Although we had access to the participants' medical records, we did not unfortunately collect complete data on immunomodulatory medications so cannot include these in analyses. We agree that this may mean that there is incomplete control for confounding, although it is reassuring that levels of statin use (which we did collect) were similar in cases (40%) and controls (39%) so this important class of medications would have been excluded as a potential confounder on the basis of the backwards stepwise logistic regression. We now highlight this as a limitation to our study in the

discussion (p10-11). As stated in the original response, we did not consider statins (or other medications) as a priori confounders because, while they may reduce the risk of hospitalisation and death due to influenza, it is not clear whether they would affect the presence of ILI – our primary exposure – rather than ILI severity. Finally it is also reassuring that our results in this study were consistent with those obtained in other work using self-controlled case series analysis (see reference 19) – a method that implicitly controls for fixed confounders (such as long-term medication use).