PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Clinical and epidemiological profile of patients with severe H1N1/09 pandemic influenza in Australia and New Zealand: a observational cohort study
AUTHORS	Cheng, Allen; Kotsimbos, Tom; Reynolds, Anna; Bowler, Simon; Brown, Simon; Hancox, Bob; Irving, Louis; Jenkins, Christine; Thompson, Philip; Simpson, Graham; Waterer, Grant; Wood-Baker, Richard; Kelly, Paul

VERSION 1 - REVIEW

REVIEWER	***Paul Ananth Tambyah*** Assoc Professor of Medicine National University of Singapore
REVIEW RETURNED	04-Mar-2011

THE STUDY	There have been a large number of descriptive studies of the H1N1 2009 pandemic including some from Australia. One of the strong features of this study was the remarkable reporting of very low overall mortality which supports the epi findings of (Muscatello et al EID 2010) but needs to be highlighted. This low mortality - zero in seasonal flu despite nearly half not receiving any antivirals at all is remarkable and should be highlighted. It would be relevant especially as a number of reports have suggested that not using antivirals in hospitalised patients with influenza will lead to dire consequences - these data show otherwise. The type of respiratory samples is not specified - this is important as Mulrenna et al (PLoS One 2010) have pointed out that in Australian patients, LRT samples were also important. The authors also do not clarify if their population included the pediatric population reported in Pediatrics 2011 (Yung et al).
RESULTS & CONCLUSIONS	These are important data and obviously collected with some effort. There are however some gems in the data which are not well highlighted
GENERAL COMMENTS	This was a good effort but the angle needs to be highlighted - low mortality - sick patients, limited use of antivirals for example

REVIEWER	***Robert Fowler*** Sunnybrook Research Institute, Sunnybrook Health Sciences Institute
REVIEW RETURNED	25-Mar-2011

THE STUDY	The primary hypothesis and questions might be more clearly stated and focused upon throughout the paper. i.e. comparison of H1N1 and seasonal influenza. There were a number of sections that seem to shift focus towards COPD and pneumonia vs. the H1N1/seasonal comparison. This detracted from the message I thought.

	Although the methods are fairly standard for a cohort study, some associations may be confounded by age and not specifically related to age-related co-morbid conditions.
RESULTS & CONCLUSIONS	As above, I think the main comparisons and message could be made more clear.
GENERAL COMMENTS	I think this is a very important topic, the comparison of seasonal vs. h1N1 flu (albeit very challenging because inherently different age cohorts were most at risk). I am glad the authors are tackling it. My main comment is that the manuscript loses its focus (I think H1N1 vs. seasonal flu) in various parts and discusses sub-aspects of COPD, pneumonia for example, that detract from the main comparison. There are a few sections where it is not completely clear the groups under comparison, but this is easily rectified. Specific Comments. Abstract Can the abstract be more clear on who was in the cohort (all patients admitted to hospital, to ICU, others). Is there a word missing here… "Patients with H1N1/09 influenza were younger, were more likely to have fever XXXXX and were more likely to be pregnant," Mortality isn't listed for seasonal influenza, only for H1N1. Background Fairly clearly written. Methods. What were the triggers for active PCR based surveillance. Were they the same between H1N1 and seasonal influenza or left to clinical discretion? My sense is that in 2009-10, younger people (with H1N1) were more actively screened for illness where older people (more likely with seasonal influenza) were less actively screened. Would be good to know that the trigger for testing for flu was the same regardless of the phenotype of flu. From the PCR based diagnostics, is it clear that all seasonal influenza was not in part H1N1 just not subtyped as H1N1/2009? I appreciate that data collection in the midst of a pandemic is challenging, however, for some of the definitions, e.g. "Pneumonia was defined as the presence of respiratory symptoms consistent with pneumonia together with radiological evidence of consolidation reported by a radiologist or site investigator." - are there any more firm components of the definition that was used to inform the pneumonia diagnosis (fever + change in sputum + WBC count + respiratory symptoms + CXR findings)? Results The following need correction: "The source of infection was known in 130 (223%) cases..." It's not clear why there is a paragraph focusing upon COPD and

	including mortality only in this subgroup under the 'Risk Factors' section. It seems to over-emphasize these points and not really in line with "risk factors". The heading "Risk Factors" might be better termed "Co-morbid conditions" as they are not really investigated as risk factors in comparison to patients without flu, from the population perspective, just a listing of common conditions. "In the 216 patients with asthma or COPD, 68 (31%) had radiologically confirmed pneumonia (compared to 39% in patients without asthma or COPD, p=0.07) and 15% were admitted to ICU (compared to 23% in other patients, p=0.015). The 30-day mortality of patients with asthma or COPD was 4%." I suspect that COPD and IHD are co-linear with age and that it is the age of patients (presumably those older had a lesser chance of H1N1 because of prior exposure to a similar strain etc. and that it is age, not COPD/IHD that are different – just more likely among older patients? It is unclear to me how "pneumonia" is being used. Did all these patients not have influenza-related pneumonia? Is this just secondary bacterial pneumonia (and if so, a clearer sense of how it is distinguished from influenza pneumonia) is important – it's not easy! – but could be based on timing of infiltrates, new culture results, etc. The OR's reported for pneumonia/non-pneumonia comparisons are univariate comparisons for this outcome? "Sputum cultures were taken in 164 patients with pneumonia;" – is it 'clinically-suspected pneumonia'? in whom cultures were taken and 23 were positive? This is a small point, but in "A higher proportion of patients with H1N1/09 influenza required admission to intensive care" – I might change required to received – often I think younger patients with H1N1 ended up getting admitted to ICU in comparison to older patients with seasonal influenza as decision as opposed to a 'requirement' (i.e. reflects decision-making rather that some absolute need) In the "Intensive care admission" paragraph, the cohort being described isn't clear to me – all patients with flu, just those with H1N1; and, the nature of the paper – comparing H1N1 to seasonal, doesn't come out clearly here. In the uni/Multivariable analysis, why was "Radiologically Confirmed pneumonia" not included? Discussion There is a lot of discussion about the limitations – which is appropriate for any observational study done in the context of a pandemic; however, the main message/findings could come out even more clearly in the lead in paragraph. Thanks very much for allowing me the opportunity to review and good luck with the next iteration.

REVIEWER	***Aubree Gordon*** Assistant Researcher

	University of California, Berkeley I have no competing interests to report.
REVIEW RETURNED	15-Apr-2011

THE STUDY	Authors need to give more information about the testing criteria, method of sample collection, and the protocol used for RT-PCR testing.
GENERAL COMMENTS	1. Please cite the protocol used for influenza testing. Was it the CDC protocol or was another(s) used? Was real-time (qRT-PCR) performed or traditional RT-PCR? Did testing procedures vary by site? Additionally, Reverse Transcriptase-Polymerase Chain Reaction is used for diagnosis of influenza. Please correct this in the paper all references to PCR should be replaced with RT-PCR. 2. State how influenza samples were collected. 3. Since the authors were in a position to evaluate RT-PCR testing, they should remove all comments related to the sensitivity of the test. Further, I am concerned about the comment about discordant results based on repeated testing. First of all how do the authors know that it is sensitivity and not specificity, or some mix of both that is responsible for the discordant results? Second, sensitivity of influenza testing is determined by two factors; the sensitivity of the sample collection method and the sensitivity of the testing method. Did the authors repeat RT-PCR testing on the same sample or did they collect additional samples? If the testing is being repeated on the same sample, than there should be extremely few discordant results. If a significant number of discordant results was detected using the same sample, than I would be suspicious of contamination or sloppy adherence to the protocol. If different samples where used in the testing, discordant results would not be surprising as samples collected at different times will likely have different quantities of virus, and the sensitivity of influenza testing is dependent on the sample collection method. 4. Since many of the co-morbidities are related to age the authors should adjust for age in the analysis of co-morbidities. 5. Please cite the statistical program used for analysis. 6. It is not necessary to include both p-values and confidence intervals in table 5. 7. Why was chest x-ray excluded in the multivariable model? It appears from table 3 that all participants had data about consolidation on CXR and the key says that NI or not included was because of missing data. Or is table 3 incorrect? 8. How was height and weight “estimated”?

VERSION 1 – AUTHOR RESPONSE

Thank you for the opportunity to respond to the issues raised by the reviewers.

Reviewer: Paul Ananth Tambyah (National University of Singapore)

There have been a large number of descriptive studies of the H1N1 2009 pandemic including some from Australia. One of the strong features of this study was the remarkable reporting of very low overall mortality which supports the epi findings of (Muscatello et al EID 2010) but needs to be highlighted. This low mortality - zero in seasonal flu despite nearly half not receiving any antivirals at all is remarkable and should be highlighted. It would be relevant especially as a number of reports have suggested that not using antivirals in hospitalised patients with influenza will lead to dire

consequences - these data show otherwise.

Response: We have added the following statement to the results “mortality was similar in patients treated with either oseltamivir or zanamivir (24 of 431; 5.6%) compared to those not treated with antiviral agents (9 of 127; 7%; p=0.53), and the following statement to the discussion “While the effectiveness of treatment cannot be assessed in this observational study, the relatively low mortality reported here, similar to those from national notifiable data (Kelly MJA 2011), were not different in patients not treated with antiviral agents”.

The type of respiratory samples is not specified - this is important as Mulrenna et al (PLoS One 2010) have pointed out that in Australian patients, LRT samples were also important.

Response: We did not collect data on the site of swab collected which was left to the discretion of the clinician. Routine clinical practice was to take upper respiratory tract specimens from non-intubated patients and both upper and lower respiratory tract specimens from intubated patients, as the data presented in the Mulrennan study was widely known in the ICU community in 2009.

The authors also do not clarify if their population included the pediatric population reported in Pediatrics 2011 (Yung et al).

Response: We did not include any specialist paediatric hospitals in this study, and only 4 of 26 paediatric patients (< 18 years) were admitted to intensive care units at participating hospitals.

These are important data and obviously collected with some effort. There are however some gems in the data which are not well highlighted. This was a good effort but the angle needs to be highlighted - low mortality - sick patients, limited use of antivirals for example

Reviewer: Robert Fowler (Sunnybrook Research Institute, Sunnybrook Health Sciences Institute)

The primary hypothesis and questions might be more clearly stated and focused upon throughout the paper. i.e. comparison of H1N1 and seasonal influenza. There were a number of sections that seem to shift focus towards COPD and pneumonia vs. the H1N1/seasonal comparison. This detracted from the message I thought…I think the main comparisons and message could be made more clear.

Response: We have restated the aims to read: “...we aimed to explore differences in risk factors, clinical features and outcome between patients with H1N1/09 influenza and other seasonal strains of influenza, and to describe the clinical features and markers of severity in hospitalized patients with influenza”

We have re-phrased the start of the discussion to read
This study describes the clinical features and outcomes of hospitalized patients with pandemic H1N1/09 influenza and seasonal strains at 9 hospitals in Australia and New Zealand. We found that patients with H1N1/09 influenza had a similar clinical presentation to those with seasonal influenza but were younger and less likely to have age-associated comorbidities. Radiological evidence of pneumonia was an important marker of severity, but although a significant proportion of patients were admitted to intensive care, overall mortality was relatively low.

Although the methods are fairly standard for a cohort study, some associations may be confounded by age and not specifically related to age-related co-morbid conditions.

Response: We fully acknowledge that this is the likely explanation for the differences in proportion with comorbidities, with the obvious exception of pregnancy. This is stated in the discussion about co-

morbidities as follows: “The differences in co-morbdities may in part reflect the younger age of patients with H1N1/09 infection.”

I think this is a very important topic, the comparison of seasonal vs. h1N1 flu (albeit very challenging because inherently different age cohorts were most at risk). I am glad the authors are tackling it.

My main comment is that the manuscript loses its focus (I think H1N1 vs. seasonal flu) in various parts and discusses sub-aspects of COPD, pneumonia for example, that detract from the main comparison. There are a few sections where it is not completely clear the groups under comparison, but this is easily rectified.

Response: We have clarified the aims and discussion above.

Abstract: Can the abstract be more clear on who was in the cohort (all patients admitted to hospital, to ICU, others).

Response: We have amended the methods to read “We performed active surveillance to define a cohort of patients hospitalized with PCR-confirmed influenza...”

Is there a word missing here? “Patients with H1N1/09 influenza were younger, were more likely to have fever XXXXX and were more likely to be pregnant,”

Response: We have corrected this to read: “Patients with H1N1/09 influenza were younger, were more likely to report a history of fever...”

Mortality isn’t listed for seasonal influenza, only for H1N1.

Response: Table 4 lists in hospital mortality as 5% and 0 for patients hospitalized with H1N1/09 influenza and other (seasonal) strains of influenza respectively

Methods: What were the triggers for active PCR based surveillance. Were they the same between H1N1 and seasonal influenza or left to clinical discretion? My sense is that in 2009-10, younger people (with H1N1) were more actively screened for illness where older people (more likely with seasonal influenza) were less actively screened. Would be good to know that the trigger for testing for flu was the same regardless of the phenotype of flu.

Response: Testing was left to the discretion of clinicians. We do not believe that early in the outbreak there would be significant biases in testing elderly patients as evidence that elderly patients were not as susceptible to disease was not widely known. However, we have included this as a potential bias in the paragraph on limitations:

Despite high levels of awareness in medical staff, clinical testing criteria were operating during the period of the study (15), and were likely to bias the proportion of patients reporting fever and respiratory symptoms. Thus, the clinical syndrome of influenza like illness is likely to be less sensitive than that described here. Further, testing for influenza was left to the discretion of clinicians and this other biases (such as more intensive testing in younger patients) may be present.

From the PCR based diagnostics, is it clear that all seasonal influenza was not in part H1N1 just not subtyped as H1N1/2009?

Response: Yes – all laboratories performing subtyping were able to differentiate H1N1/09 influenza from seasonal H1N1 strains.

I appreciate that data collection in the midst of a pandemic is challenging, however, for some of the definitions, e.g. “Pneumonia was defined as the presence of respiratory symptoms consistent with pneumonia together with radiological evidence of consolidation reported by a radiologist or site investigator.” - are there any more firm components of the definition that was used to inform the pneumonia diagnosis (fever + change in sputum + WBC count + respiratory symptoms + CXR findings)?

Response: We have corrected the definition to read “Pneumonia was defined as the presence of acute respiratory symptoms consistent with pneumonia together with radiological evidence of consolidation, in the absence of an alternative diagnosis.” These definitions are in line with other studies of community-acquired pneumonia, such as the Australian Community Acquired Pneumonia Study (Charles CID 2008; 46: 1514)

Results: The following need correction: “The source of infection was known in 130 (223%) cases...”

Response: This has been corrected to “...130 (23.2%) of cases...”

It’s not clear why there is a paragraph focusing upon COPD and including mortality only in this subgroup under the ‘Risk Factors’ section. It seems to over-emphasize these points and not really in line with “risk factors”. The heading “Risk Factors” might be better termed “Co-morbid conditions” as they are not really investigated as risk factors in comparison to patients without flu, from the population perspective, just a listing of common conditions.

“In the 216 patients with asthma or COPD, 68 (31%) had radiologically confirmed pneumonia (compared to 39% in patients without asthma or COPD, p=0.07) and 15% were admitted to ICU (compared to 23% in other patients, p=0.015). The 30-day mortality of patients with asthma or COPD was 4%.”

Response: We have changed the subheading and the caption of table 2 to read “co-morbidities” as suggested. We have added a comment regarding outcomes in other risk groups: “In the 45 patients that were pregnant, 19 (42%) had radiologically confirmed pneumonia, 17 (38%) were admitted to ICU and 1 patient (2%) died”

I suspect that COPD and IHD are co-linear with age and that it is the age of patients (presumably those older had a lesser chance of H1N1 because of prior exposure to a similar strain etc. and that it is age, not COPD/IHD that are different – just more likely among older patients?

Response: We agree that this is the most likely explanation for the differences in proportion with comorbidities. This is stated as follows “The differences in co-morbidities may in part reflect the younger age of patients with H1N1/09 infection.”

It is unclear to me how “pneumonia” is being used. Did all these patients not have influenza-related pneumonia? Is this just secondary bacterial pneumonia (and if so, a clearer sense of how it is distinguished from influenza pneumonia) is important – it’s not easy! – but could be based on timing of infiltrates, new culture results, etc. The OR’s reported for pneumonia/non-pneumonia comparisons

are univariate comparisons for this outcome?

Response: We did not find it possible to differentiate reliably between primary influenza pneumonitis and secondary bacterial pneumonia in this study, unless cultures were positive for a bacterial pathogen. We have used the term pneumonia to mean radiological evidence of consolidation in patients with clinical symptoms and signs consistent with a respiratory infection, of bacterial and/or viral aetiology.

"Sputum cultures were taken in 164 patients with pneumonia;" – is it 'clinically-suspected pneumonia'? in whom cultures were taken and 23 were positive?

Response: As above, we use the term pneumonia to mean radiological evidence of consolidation in patients with clinical symptoms and signs consistent with a respiratory infection.

This is a small point, but in "A higher proportion of patients with H1N1/09 influenza required admission to intensive care" – I might change required to received – often I think younger patients with H1N1 ended up getting admitted to ICU in comparison to older patients with seasonal influenza as decision as opposed to a 'requirement' (i.e. reflects decision-making rather that some absolute need)

Response: This has been changed to "A higher proportion of patients with H1N1/09 influenza were admitted to intensive care".

In the "Intensive care admission" paragraph, the cohort being described isn't clear to me – all patients with flu, just those with H1N1; and, the nature of the paper – comparing H1N1 to seasonal, doesn't come out clearly here.

Response: We looked at all factors that were associated with ICU admission, including strain type, in all patients with influenza admitted to hospital. We have clarified this in the first sentence as follows: "A higher proportion of patients with H1N1/09 influenza were admitted to intensive care compared to those admitted with seasonal strains"

In the uni/Multivariable analysis, why was "Radiologically Confirmed pneumonia" not included?

Response: We felt that pneumonia is an intermediate variable in the causal pathway to severe illness and this to include this in the multivariate model would result in an "overadjustment" bias. (Schisterman EF et al, Overadjustment Bias and Unnecessary Adjustment in Epidemiologic Studies. Epidemiology 2009 20(4) 488-495)

Discussion: There is a lot of discussion about the limitations – which is appropriate for any observational study done in the context of a pandemic; however, the main message/findings could come out even more clearly in the lead in paragraph.

Response: See response above – we now state the main findings at the start of the discussion.

Reviewer: Aubree Gordon (University of California, Berkeley)

Authors need to give more information about the testing criteria, method of sample collection, and the

protocol used for RT-PCR testing.

1. Please cite the protocol used for influenza testing. Was it the CDC protocol or was another(s) used? Was real-time (qRT-PCR) performed or traditional RT-PCR? Did testing procedures vary by site? Additionally, Reverse Transcriptase-Polymerase Chain Reaction is used for diagnosis of influenza. Please correct this in the paper all references to PCR should be replaced with RT-PCR.

Response: All laboratories involved used the CDC real time reverse transcriptase polymerase chain reaction (rRT-PCR) protocol. We have amended PCR to read rRT-PCR as recommended.

2. State how influenza samples were collected.

Response: We did not collect data on the site of swab collected which was left to the discretion of the clinician. We have added the following: “Clinical specimens were taken at the discretion of the clinician. Common clinical practice in Australia is that an upper respiratory tract specimen (a combined nose throat swab) was taken in non-intubated patients and both upper and lower respiratory tract specimens were taken in intubated patients”

3. Since the authors were in a position to evaluate RT-PCR testing, they should remove all comments related to the sensitivity of the test. Further, I am concerned about the comment about discordant results based on repeated testing. First of all how do the authors know that it is sensitivity and not specificity, or some mix of both that is responsible for the discordant results? Second, sensitivity of influenza testing is determined by two factors; the sensitivity of the sample collection method and the sensitivity of the testing method. Did the authors repeat RT-PCR testing on the same sample or did they collect additional samples? If the testing is being repeated on the same sample, than there should be extremely few discordant results. If a significant number of discordant results was detected using the same sample, than I would be suspicious of contamination or sloppy adherence to the protocol. If different samples where used in the testing, discordant results would not be surprising as samples collected at different times will likely have different quantities of virus, and the sensitivity of influenza testing is dependent on the sample collection method.

Response: We did not collect data on the site of specimen collection or the quality of specimen. All laboratories serving the hospitals involved in this study undergo quality control procedures, including the use of internal controls. We acknowledge that the statement referring to discordant results is anecdotal; we have removed this statement and referenced a paper that discusses this issue in further detail (Mulrennan PLoS One. 2010; 5(9): e12849). This sentence now reads “Nucleic acid detection using PCR is regarded as highly sensitive and specific for the diagnosis of influenza, but recently published data suggests that the sensitivity of upper respiratory tract sampling (which most non-intubated patients receive) may not be optimal in patients with lower respiratory tract involvement.”

4. Since many of the co-morbidities are related to age the authors should adjust for age in the analysis of co-morbidities.

Response: We only performed an unadjusted comparison of the proportions reporting comorbidities, and fully acknowledge that age is the most likely explanation for the differences as follows: “The differences in co-morbdities may in part reflect the younger age of patients with H1N1/09 infection.”

In exploring the predictors of ICU admission, age was not associated with ICU admission and was dropped from the final model; forcing age into the model changes the magnitude of effect of other comorbidities to a minor degree.

Table: Factors associated with ICU admission

Odds Ratio Std. Err. z P>z [95% Conf. Interval]
H1N1 vs seasonal 1.679032 .6121997 1.42 0.155 .8216722 3.430991
Pregnancy 2.289316 .7977299 2.38 0.017 1.156373 4.532247
Chr liver disease 2.75453 1.071644 2.60 0.009 1.284959 5.904806
COPD .5223551 .1887347 -1.80 0.072 .2572848 1.060517
Age (yrs) .9980382 .0061298 -0.32 0.749 .986096 1.010125

5. Please cite the statistical program used for analysis.

Response: We used Stata 10 for Windows (Statacorp, College Station, Texas, United States) for all analyses.

6. It is not necessary to include both p-values and confidence intervals in table 5.

Response: Statistical practices vary; we are happy to confirm to journal guidelines regarding the presentation of confidence intervals and p values.

7. Why was chest x-ray excluded in the multivariable model? It appears from table 3 that all participants had data about consolidation on CXR and the key says that NI or not included was because of missing data. Or is table 3 incorrect?

Response: See response above. We felt that pneumonia is an immediate cause in the causal pathway to severe illness and this to include this in the multivariate model would be an "overadjustment" error.

8. How was height and weight "estimated"?

Response: Patient heights and weight were obtained from the medical record or from patient report. This methods have been amended to reflect this "We defined severe obesity as a body mass index of >35 kg/m2, with height and weight obtained from the medical record or from patient self-report"

We have also added one citation that was listed as being in press.

Thank you for the opportunity to respond to your reviewers comments. Should you have any further queries, please contact the corresponding author, Allen Cheng at allen.cheng@monash.edu

VERSION 2 - REVIEW

REVIEWER	***Aubree Gordon***
REVIEW RETURNED	16-May-2011

GENERAL COMMENTS	Reviewer completed checklist only. No further comments were made.