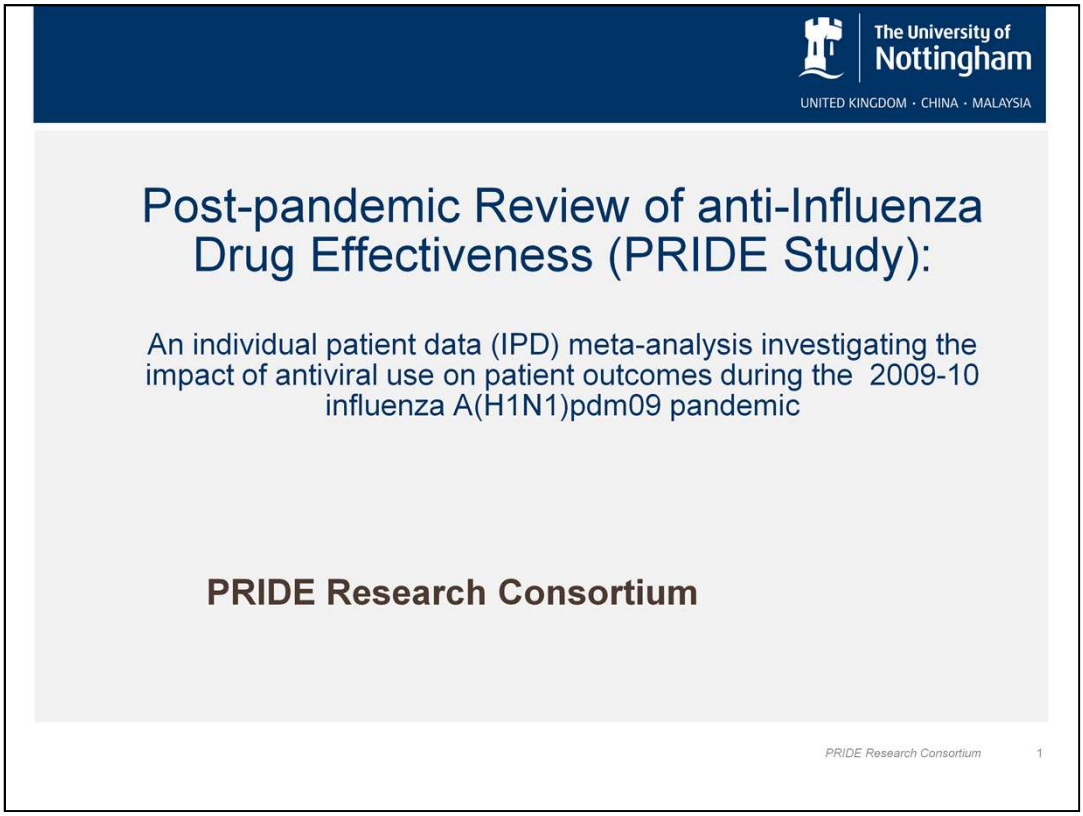
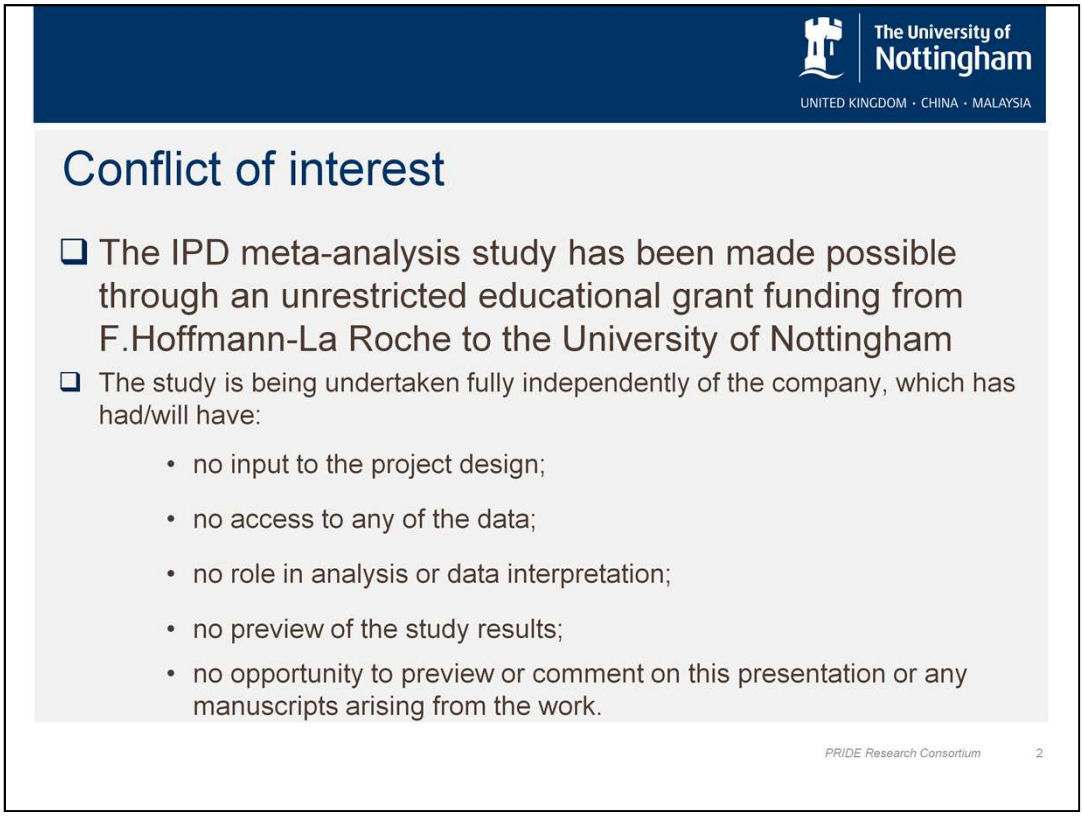
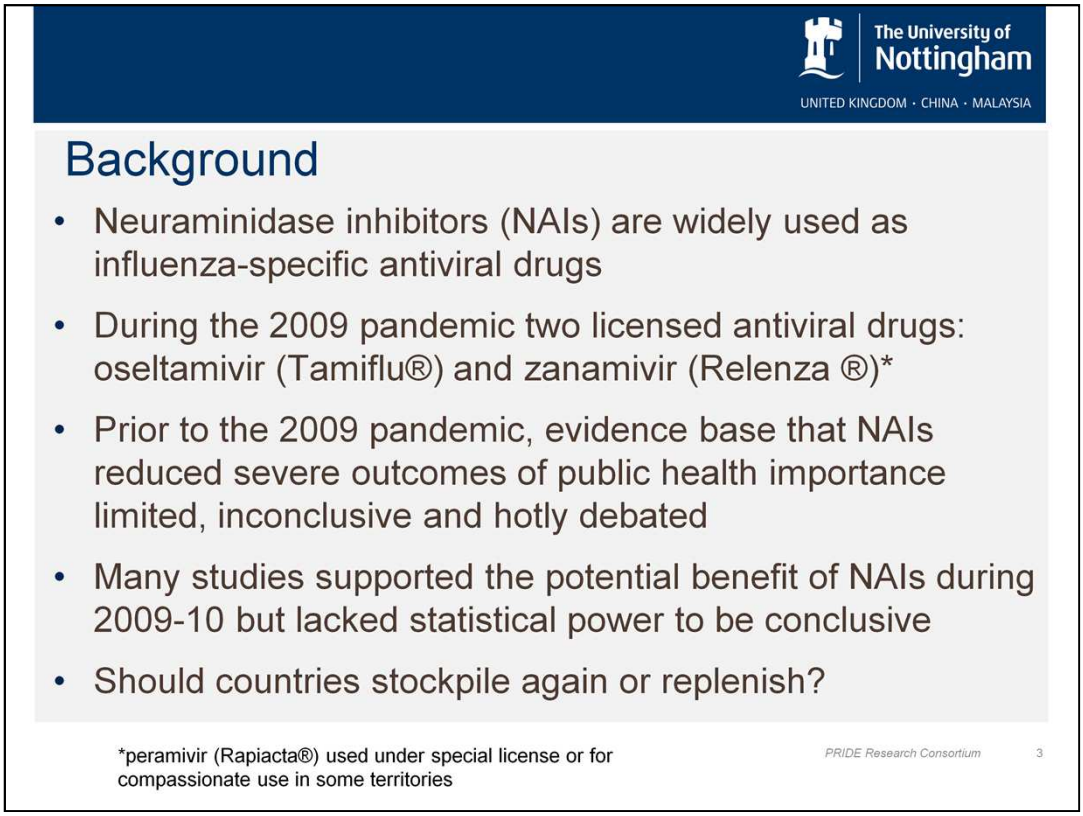


This presentation reports on the findings from the PRIDE study which is a post-pandemic review of anti-influenza drug effectiveness. Essentially we conducted an IPD analysis which involved a meta-analysis of pooled individual patient data contributed by research groups within the PRIDE Research Consortium. The PRIDE Research Consortium is a global consortium made up of 80 research groups. The data standardisation and analyses were conducted by Drs. Stella Muthuri and Sudhir Venkatesan, Research Fellows at the University of Nottingham in the UK under the strategic leadership of Professor Jonathan Van-Tam. Dr. Puja Myles provided the methodological lead on the study with statistical advice provided by Dr Jo Leonardi-Bee who heads the systematic review research group at Nottingham and is also the former statistical editor of the Cochrane skin group.

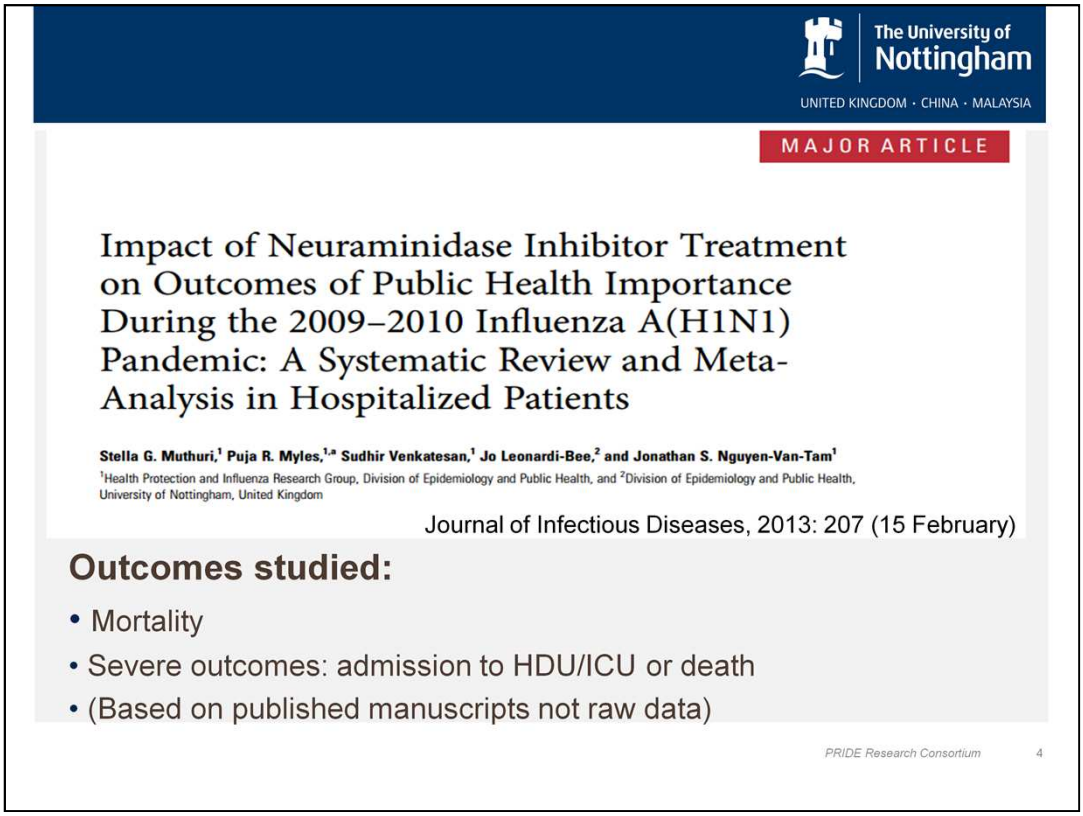


The unrestricted educational grant was awarded to Dr Puja Myles and Professor Jonathan Nguyen-Van-Tam for research in the area of pandemic influenza. Part of this grant was used to employ two Research Fellows to help with the data collation, standardisation, pooling and analysis for the PRIDE study. The full grant contract (with sensitive financial details redacted) can be viewed at http://www.nottingham.ac.uk/research/groups/healthprotection/projects/pride.aspx. This grant was not used to fund any data collection activities by the PRIDE Research Consortium Investigators. The conduct of the study has been completely independent of F. Hoffman La Roche and none of the study methods or findings were disclosed to them prior to their availability in the public domain via conference presentations or publication in peer-reveiwed academic journals.

For further declarations of interest by individual PRIDE Research Consortium Investigators, please view the declarations at the end of the published paper: http://www.thelancet.com/journals/lanres/article/PIIS2213-2600(14)70041-4/fulltext



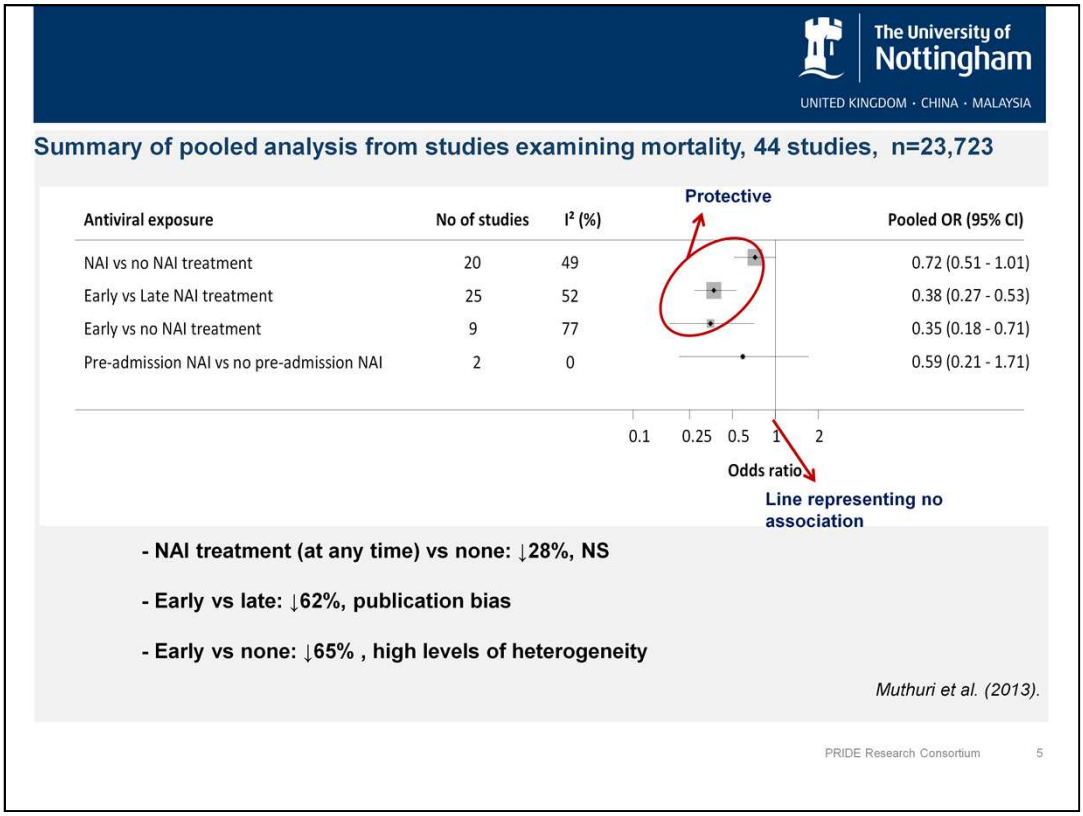
Neuraminidase inhibitors (NAIs) are widely used as influenza-specific antiviral drugs. During the 2009 pandemic two licensed antiviral drugs were available: oseltamivir (Tamiflu®) and zanamivir (Relenza ®). Peramivir ((Rapiacta®) was used under special license or for compassionate use in some territories. Prior to the 2009 pandemic, the evidence base that NAIs reduced severe outcomes of public health importance was limited, inconclusive and hotly debated. Many studies published during and after the 2009 pandemic supported the potential benefit of NAIs during 2009-10 but lacked the statistical power to be conclusive. Therefore the key policy question remained unanswered i.e. should countries stockpile again or replenish?



To try and resolve this unanswered policy question the research group at Nottingham conducted a systematic review of published manuscripts that looked at the impact of NAI antivirals on patient outcomes during the 2009 pandemic. We considered two main outcomes: mortality and admission to critical care.

Note: A random effects meta-analysis was conducted which allows for differences in the treatment effect from study to study.

This paper reported on a systematic review of published studies and was the driver for the PRIDE IPD meta-analysis. This paper can be found here: http://jid.oxfordjournals.org/content/early/2012/11/28/infdis.jis726.abstract



This slide summarises the pooled results from studies examining mortality. We considered 4 different exposure definitions for antiviral use. This figure is modelled on a forest plot. The line in the middle represents a pooled OR of 1 which means no effect. Anything to the left of this line represents a protective effect and anything to the right represents an increase in mortality. Each point estimate of relative risk is bounded by a line- the length of the line reflects the 95% confidence interval and where this line crosses the midline, it represents a statistically non-significant result.

When you consider NAI treatment at anytime versus no NAI treatment, the pooled OR shows a non-significant 28% reduction in mortality. The study then looked at early treatment i.e. treatment administered within 2 days of symptom onset. When early treatment was compared to late treatment, there was a significant 62% reduction in mortality but some evidence of publication bias (i.e. some 'missing' publications which may have shown alternative results; for more information on publication bias, please refer to the FAQs on the PRIDE study website titled 'key questions relating to the manuscript'). When early treatment was compared to no treatment the study found a 65% significant reduction in mortality but high levels of heterogeneity in the studies. The study did not find a statistically significant association between preadmission antiviral use and mortality but there were only 2 studies in this category.

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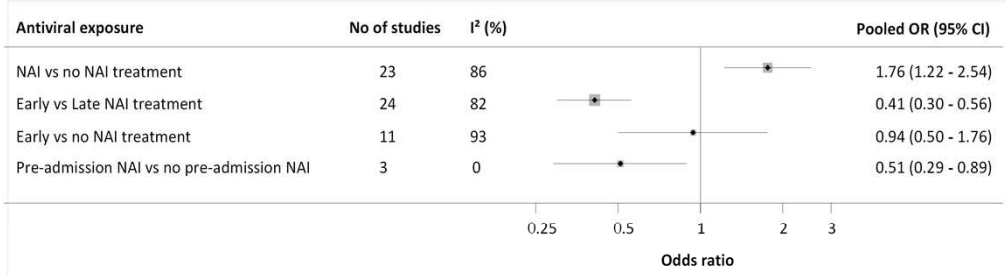
Summary of pooled analysis from studies examining severe outcome, 52 studies, n= 31,428

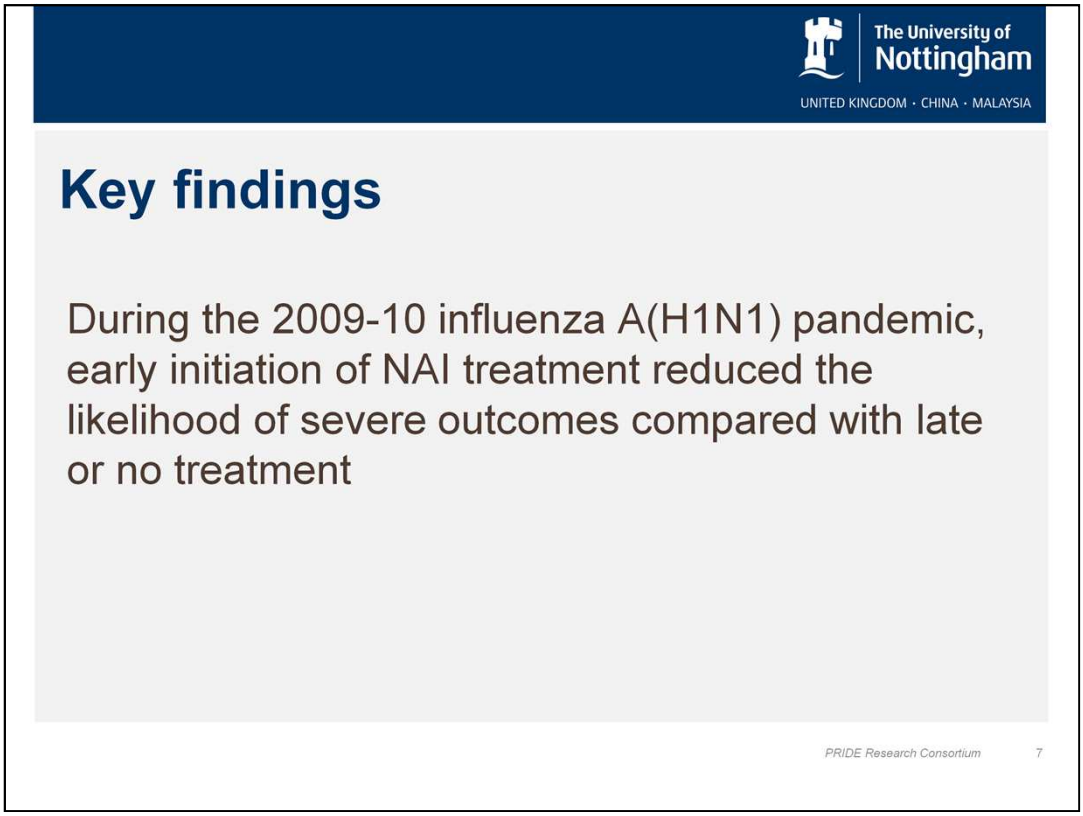


- **NAI treatment (at any time) vs none: ↑ 76%**
- **Early vs late: ↓59%**
- **Early vs none: ↓6%, NS**
- **Overall, high degree of heterogeneity**

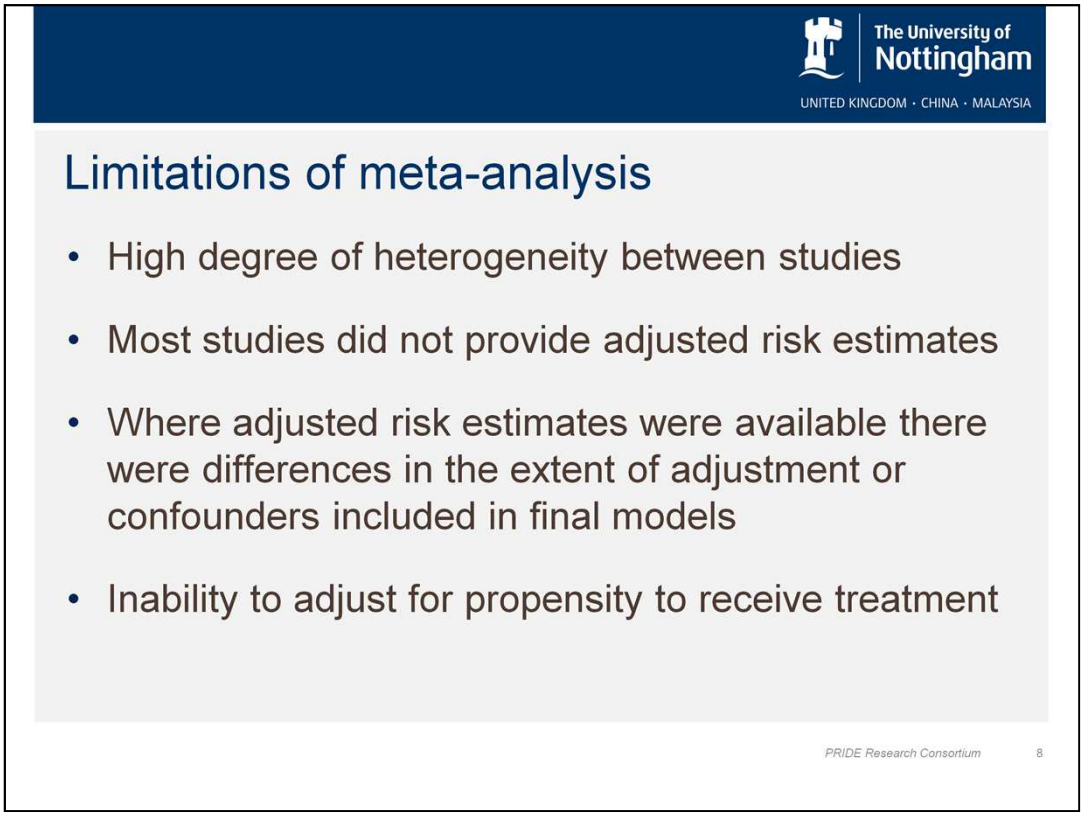
Muthuri et al. (2013).

PRIDE Research Consortium 6

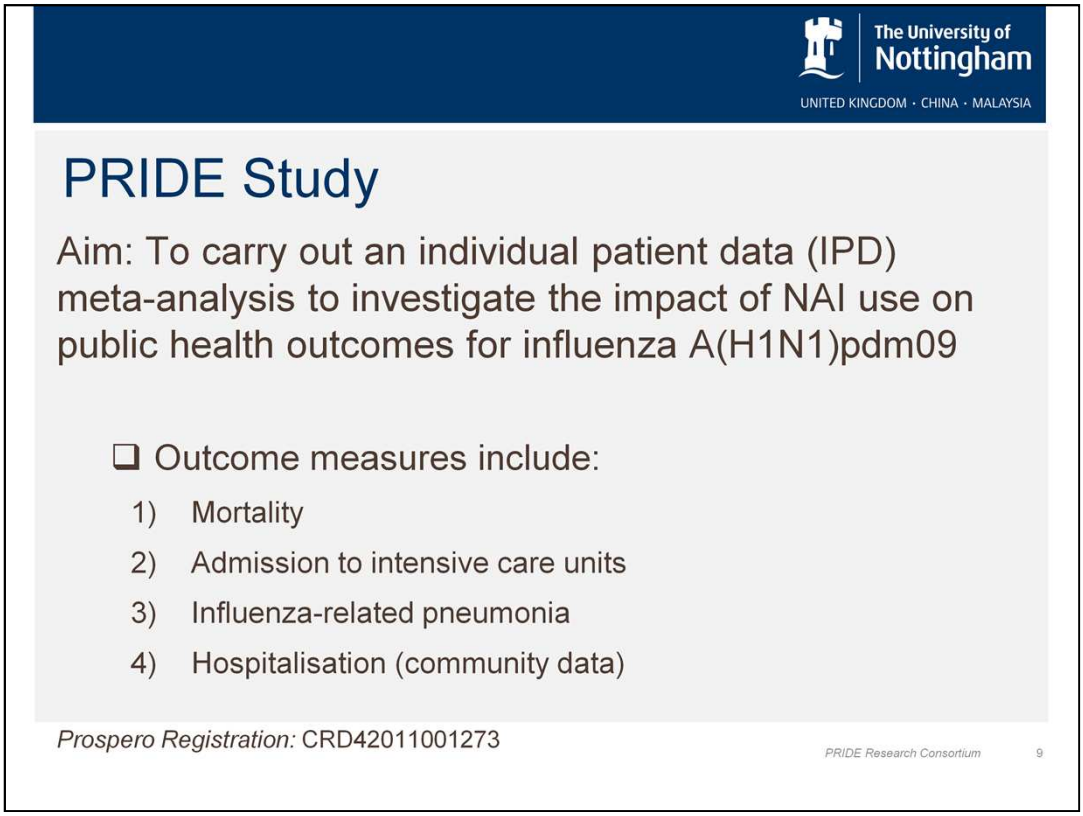
The study then looked at the impact of NAI use on severe outcomes. 'Severe outcome' was defined as admission to critical care or death. In this figure you can see that there was a statistically significant decrease of nearly 60% in severe outcomes when early treatment was compare to late treatment. There was also a statistically significant decrease in severe outcomes by nearly 50% with preadmission antiviral use. There was a statistically significant increase of more than 75% in severe outcomes when NAI use at anytime was compared to no NAI use. This isn't surprising as there is a strong likelihood that this association was confounded by underlying disease severity. This is the classic problem of confounding by indication in observational studies where treatment propensity or likelihood is not comparable in treated and non-treated patients.



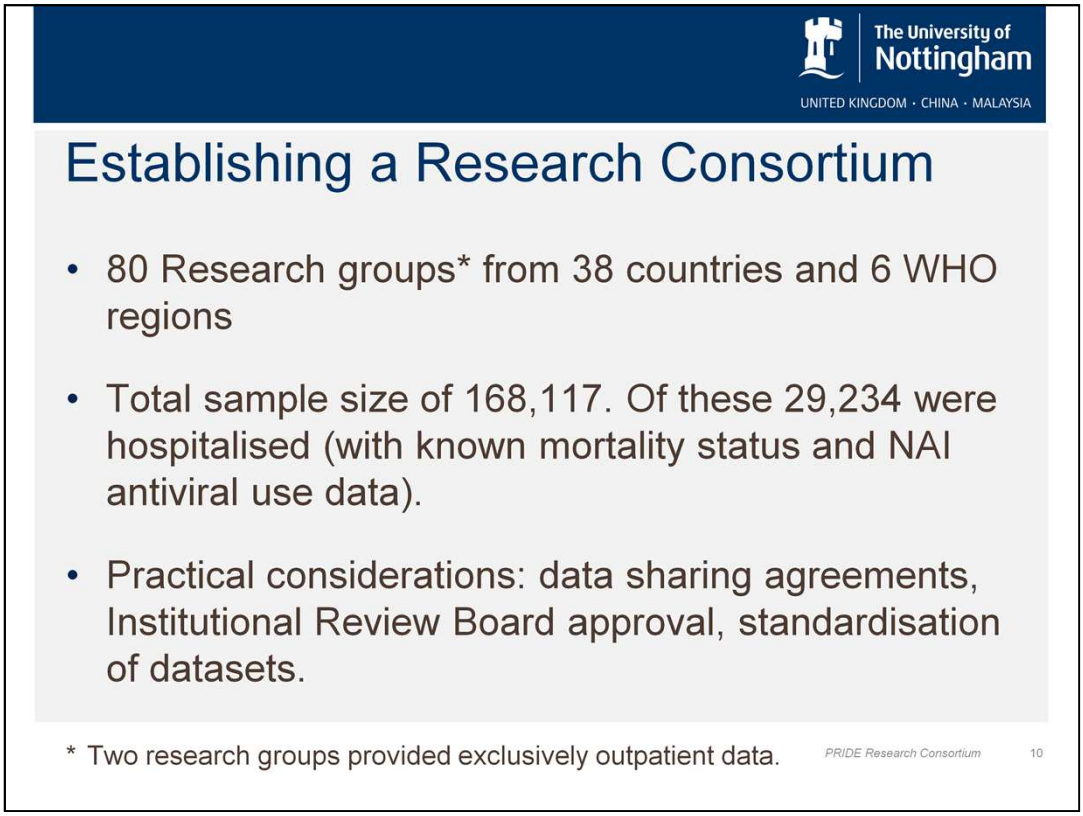
The key finding reported on the basis of this work was that during the 2009-10 influenza A(H1N1) pandemic, early initiation of NAI treatment reduced the likelihood of severe outcomes compared with late or no treatment.



There were a number of limitations with this study. There was a high degree of heterogeneity between studies in terms of the populations studied. Most studies did not provide adjusted risk estimates and where adjusted risk estimates were available there were differences in the extent of adjustment or confounders included in final models. Finally, because the study was dealing with summary data from published manuscripts, the researchers were unable to adjust for treatment propensity.



This was the driver for carrying out an individual patient data meta-analysis to further investigate the same question as part of the PRIDE study. The PRIDE study considered three outcome measures: mortality, admission to critical care and influenza-related pneumonia. This presentation will only report on the findings relating to mortality.



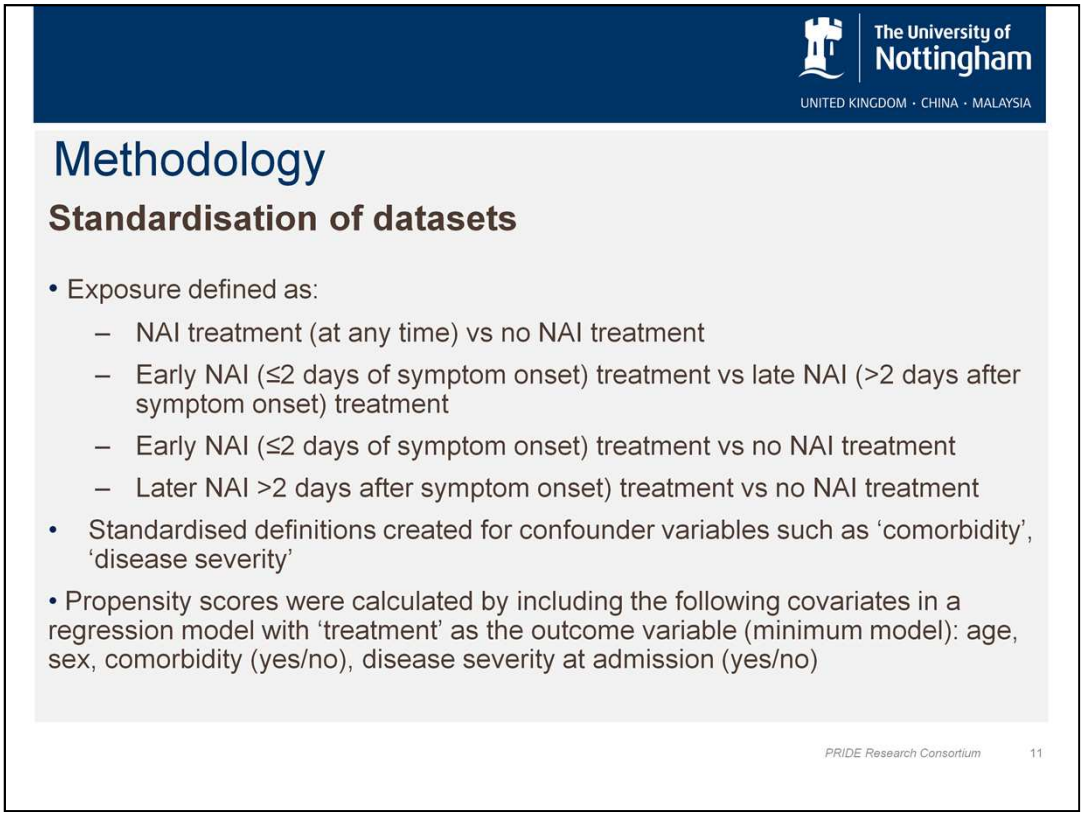
When the systematic review of published studies was conducted, the search identified 401 research groups that may have had access to patient surveillance data during the pandemic. This included letters and general reports of the pandemic experience.

The Nottingham University group wrote to the corresponding authors of these publications and received 128 (32%) replies. 51 (nearly 13%) declined (36 had no data, 3 agreed but later withdrew, 12 agreed but did not share data). The reasons quoted for refusal included lack of capacity for data extraction or restrictive institutional review board protocols that did not allow for sharing of individual level data. In some cases we found a number of publications relating to the same dataset. We also identified 2 additional research groups through collaborators and experts in the field at a later stage who contributed their data.

In the end, 80 research groups from 38 countries and 6 WHO regions joined the PRIDE Research Consortium and contributed their datasets for the analysis. Our total sample size was over 167,000 patients and of these, nearly 30,000 were hospitalised patients with known mortality and NAI antiviral treatment status.

Practical considerations when establishing the research consortium included setting up data sharing agreements, obtaining institutional review board approval and data standardisation. It took about one and a half years to get to the point of

being able to pool and analyse these data.



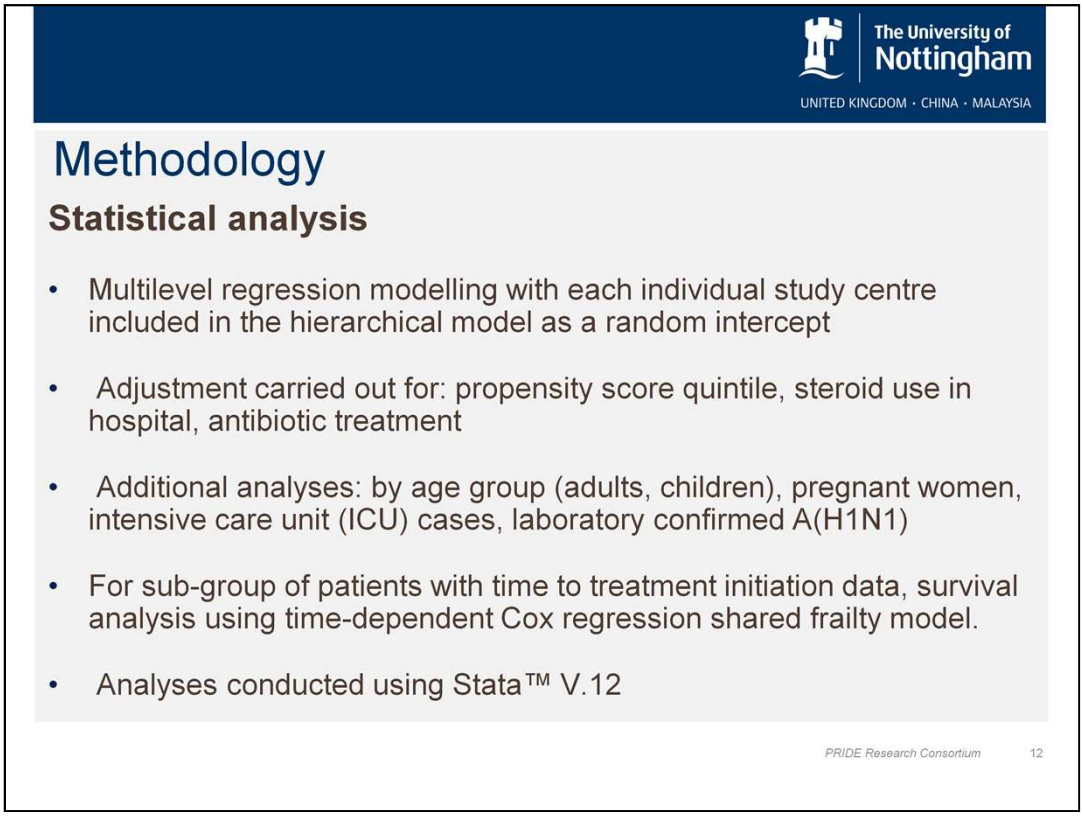
The first task for the PRIDE study investigators was data standardisation. Exposure to NAIs was defined in three ways as binary outcome measures as described earlier for the systematic review of published studies: NAI treatment (at any time) versus no NAI treatment, Early NAI treatment versus late NAI treatment and Early NAI treatment versus no NAI treatment.

Standardised definitions were created for confounder variables such as 'comorbidity' (yes/no) and disease severity (yes/no). The following conditions were counted as comorbidities of interest: asthma, COPD, other chronic lung disease, heart disease, cerebrovascular disease (not including uncomplicated hypertension), chronic liver disease, chronic renal disease, diabetes, neurological disease (including neurodevelopmental disorders), lymphoma, leukaemia, other malignancy, immunosuppression (includes patients undergoing chemotherapy leading to immunosuppression; asplenia or splenic dysfunction and HIV infection at all stages).

Disease severity assessed based on factors such as severe respiratory distress, shortness of breath, symptom scores, severity measures like AVPU etc.

Propensity scores were calculated for each individual study for each exposure definition by including factors that could influence treatment in a regression model with ' treatment' as the outcome variable. The minimum model included

age, sex, comorbidity as a binary categorical variable and disease severity at admission (again as a binary categorical variable). In addition, for datasets where additional information was present, an extended model was constructed including the following covariates as available: obesity, smoking, pregnancy, asthma, COPD, lung disease, heart disease, immunosuppression, neurological disease, renal disease and diabetes. The propensity scores were then categorised into quintiles for each individual study. Studies where exposure status to NAI at any time did not differ (because all had the treatment or all but very few i.e. one patient was not treated with antivirals) could not be included in the multilevel analysis due to the obvious cluster effect with regard to treatment.



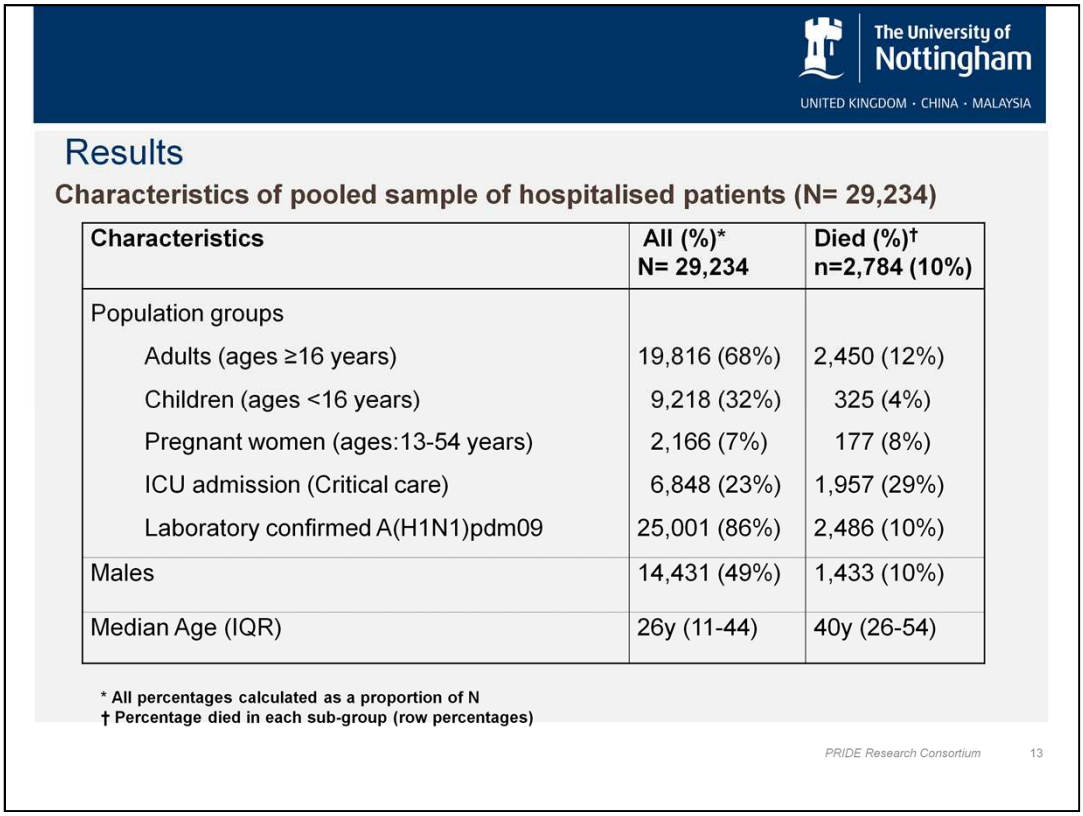
For the statistical analysis the researchers used multilevel regression modelling to take into account any clustering of effects by study centre. The researchers included each individual study centre as a random intercept which allows for the study level average mortality to be unequal across studies. The analysis included propensity score quintiles, steroid use in hospital and antibiotic treatment in the multilevel regression model.

The researchers carried out further stratified analyses by age-group (i.e. adults and children), pregnant women, ICU patients and diagnosis. All analyses were conducted in Stata version 12.

Note: The researchers used a mixed effects model (xtmelogit command in Stata) which includes random effects terms in the equation and assumes that individuals deviate randomly from the average (fixed) response. Mixed effects models assume that correlation of observations within a cluster arises from the common random effects of the cluster. An advantage of mixed effects models is that they require no minimum sample size for a particular group (Katz, 2006).

Random effects models are generally used if the levels of the independent variable are thought to be a small subset of the possible values which one wishes to generalise to and will probably produce larger standard errors (less powerful).

The researchers included each study centre as a random intercept, i.e. the intercept is assumed to vary across groups (clusters). This means the study for the group (cluster) average for the dependent variable to be unequal across groups (clusters). On the other hand, fixed or ‘non-varying’ intercepts would imply the group average for the dependent variable is assumed to be equal in each group.

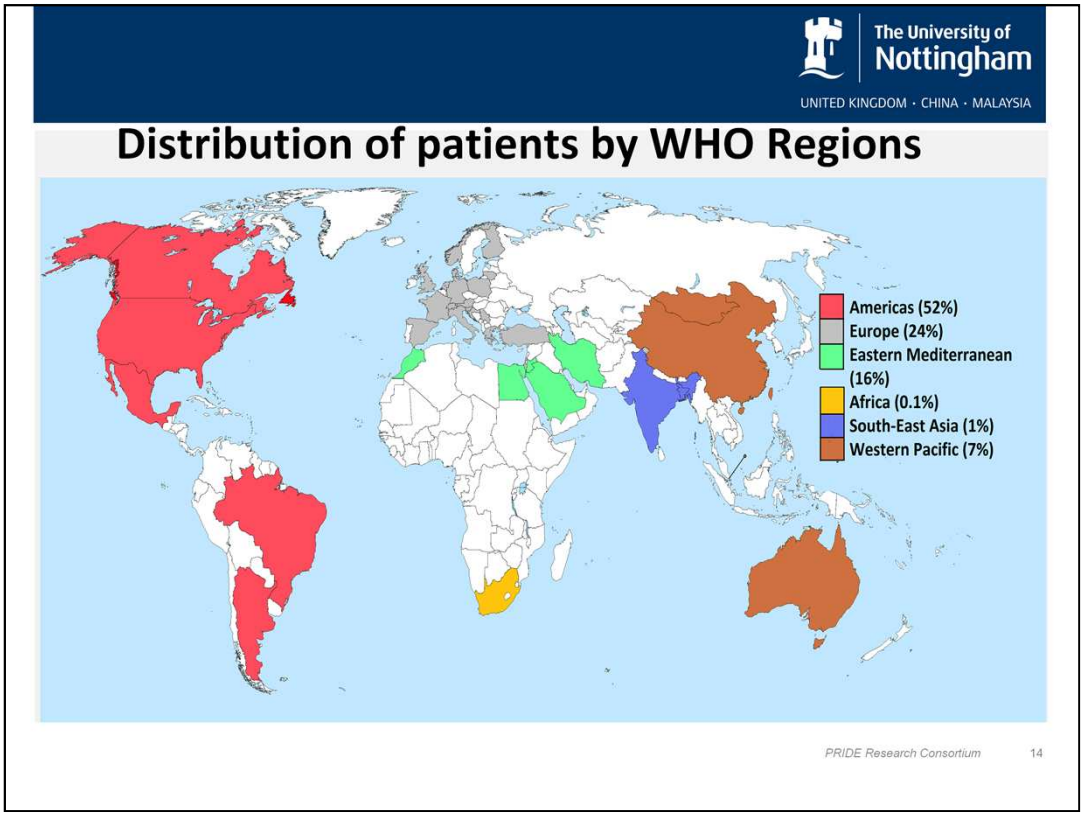


Characteristics of the pooled sample of hospitalised patients

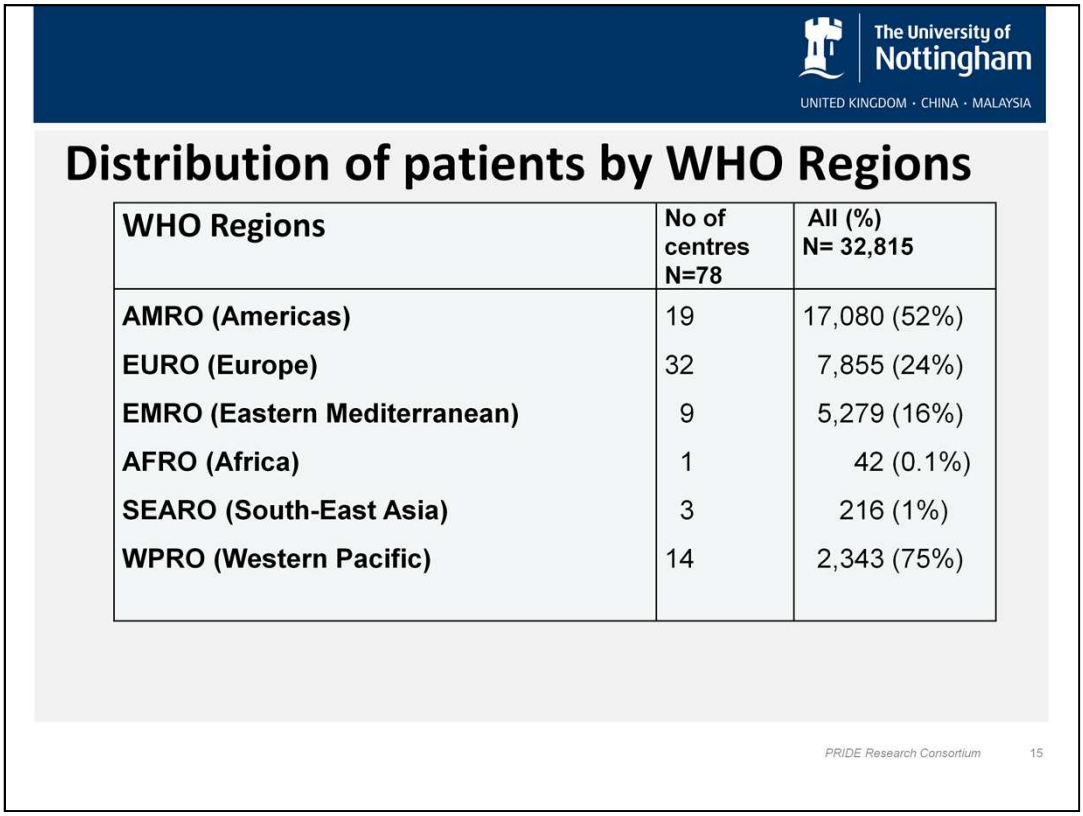
We had a total of nearly 30,000 patients in the pooled dataset. The second column shows percentages calculated as a proportion of the total sample and are not mutually exclusive. Over 85% of our pooled sample comprised laboratory confirmed A(H1N1)pdm09 cases. Nearly 70% of our sample was aged 16 years and above. 7% of our sample comprised pregnant women of all ages and about a fifth of the sample comprised patients admitted to critical care facilities.

In the column labelled ‘died’ all percentages relate to the proportion of deaths in each sub-group. Please bear in mind that these are crude death rates and do not take into account difference in illness severity of other patient characteristics. 10% of the total sample died. By sub-groups, the highest proportion of deaths (29%) was seen in patients admitted to critical care. 4% of the children in our sample died. For other sub-groups the percentage mortality ranges from 8-12%.

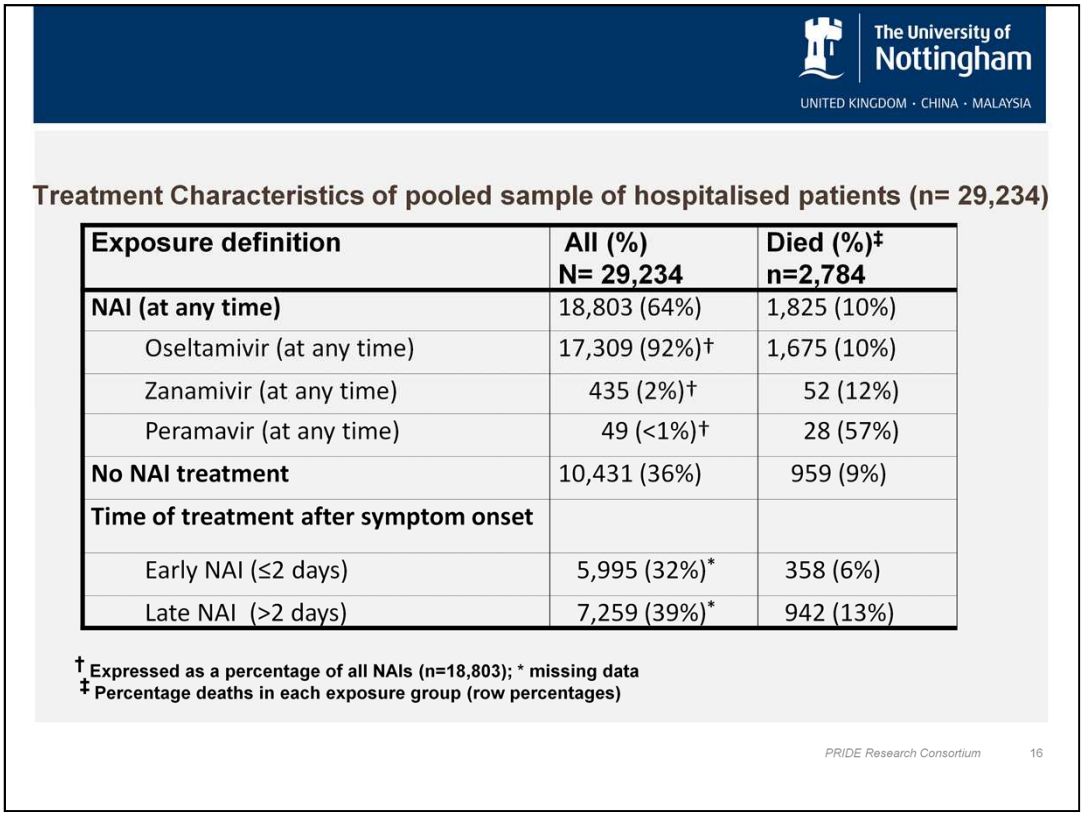
We had almost equal numbers of males and females. The median age for the total sample was 26 years and for those who died it was 40 years.



This slide shows the distribution of patients by WHO Regions. Over half of the pooled sample comprises patients from the Americas with nearly a quarter from Europe.



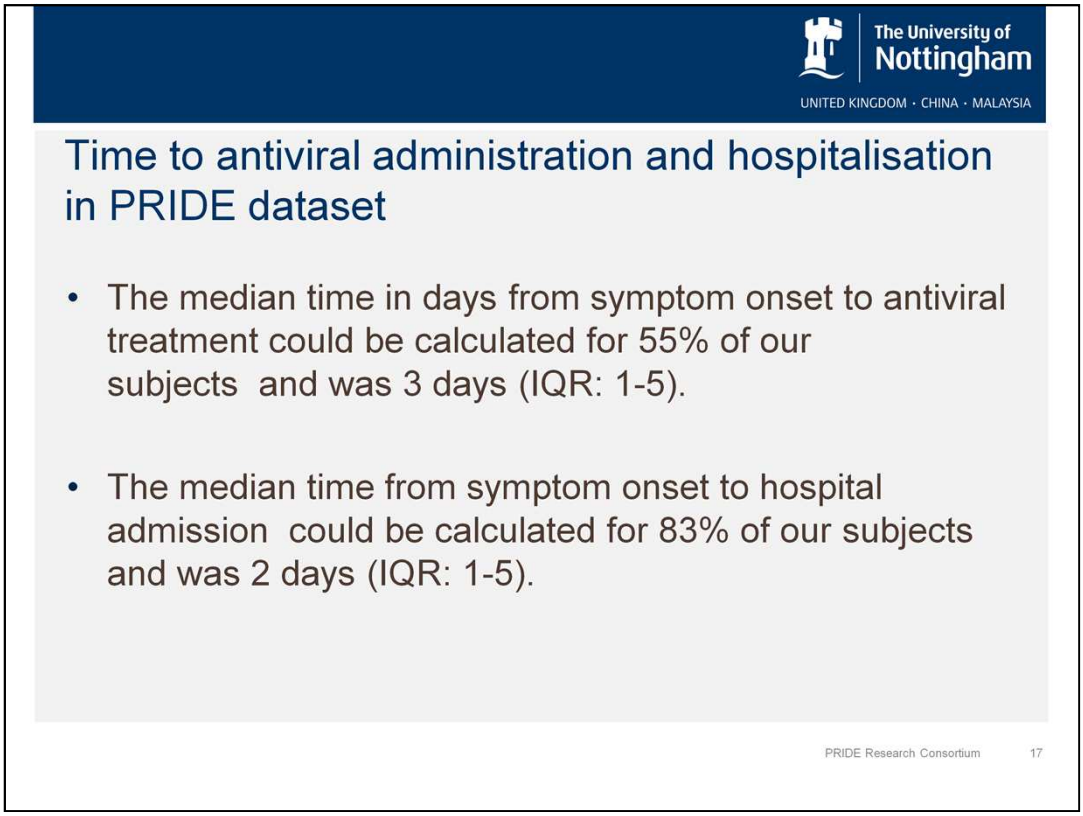
In terms of the number of study centres by WHO regions, most of the centres were from Europe followed by the Americas and the Western Pacific.



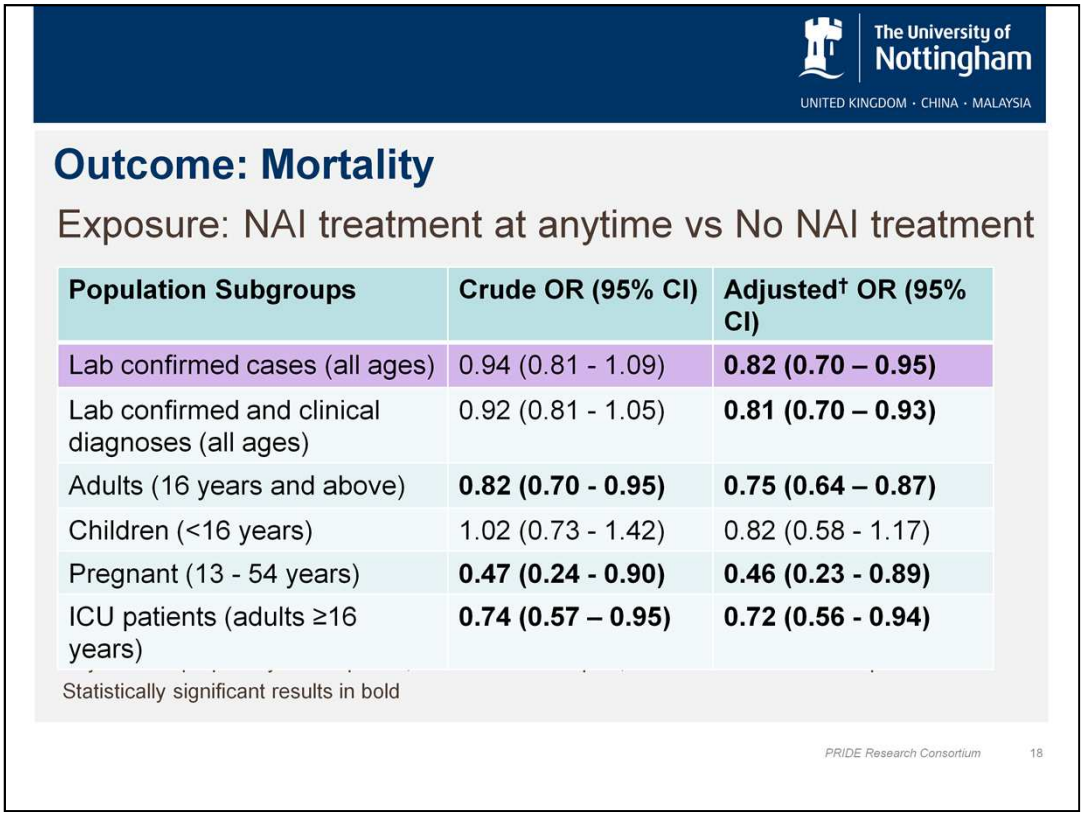
This table shows that 64% of our total sample were prescribed NAIs at any given time during hospitalisation. 92% of the total sample were prescribed oseltamivir, 2% were prescribed zanamivir and <1% were prescribed peramivir.

32% of those prescribed NAIs received these within 2 days of symptom onset and 39% received these more than 2 days after symptom onset. For 29% of those prescribed NAIs we were unable to calculate time of administration in relation to symptom onset.

The last column shows the percentage of deaths for each exposure definition. The percentages for peramivir and late NAI use especially should be interpreted with caution as these are crude death estimates that do not account for underlying disease severity.



The median time in days from symptom onset to antiviral treatment could be calculated for 55% of our subjects and was 3 days (IQR: 1-5). The median time from symptom onset to hospital admission could be calculated for 83% of our subjects and was 2 days (IQR: 1-5).

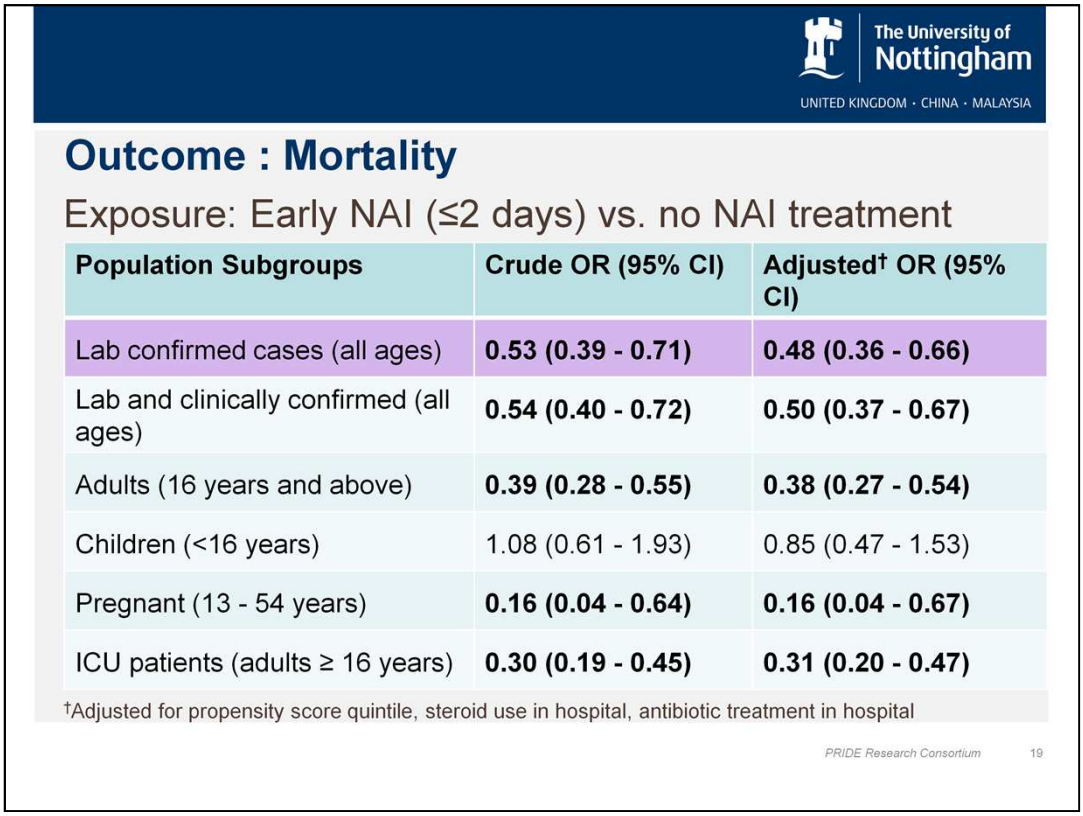


This slide shows the IPD results for the exposure definition 'NAI treatment at any time versus no NAI treatment'. Statistically significant results are in bold. The crude odds ratios (ORs) are presented for interest but the key results to focus on are the adjusted ORs in the last column.

The overall results for laboratory confirmed A(H1N1)pdm09 cases are highlighted in pink and show a statistically significant 18% reduction in mortality. If we consider both laboratory confirmed and clinically diagnosed cases, the point estimate is practically unchanged (this is important because it suggests that at the population level, there is some margin for error with regard to diagnostic accuracy of influenza in a pandemic situation; in any case, it is well recognised that in a high disease prevalence scenario, the positive predictive value associated with clinical diagnosis is increased). In adults and patients admitted the critical care there was a significant reduction of 25 and 28% respectively. In pregnant women a significant reduction of 54% was seen. The only sub-group in which no statistically significant reduction in mortality was observed was children; this does not necessarily suggest that NAI antivirals don't work in children (the point estimate suggests an 18% reduction in mortality) but that the PRIDE study was unable to provide conclusive evidence on the likely impact of NAI antivirals in children.

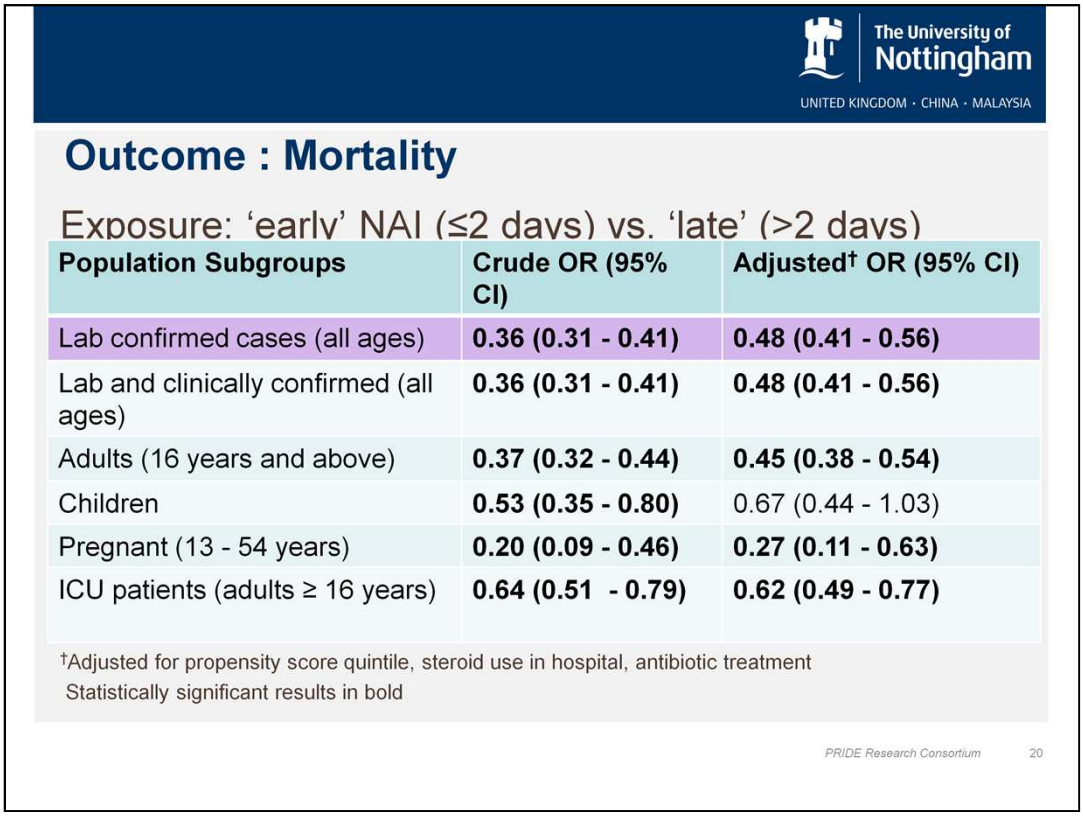
The PRIDE study categorised all individuals <16 years as children but sensitivity analyses using different age-thresholds (<1 year and <5 years) similarly failed to

find statistically significant associations between NAI antiviral use and mortality in children. These sensitivity analyses findings have been reported in Supplementary Appendix 8 accompanying the published paper.



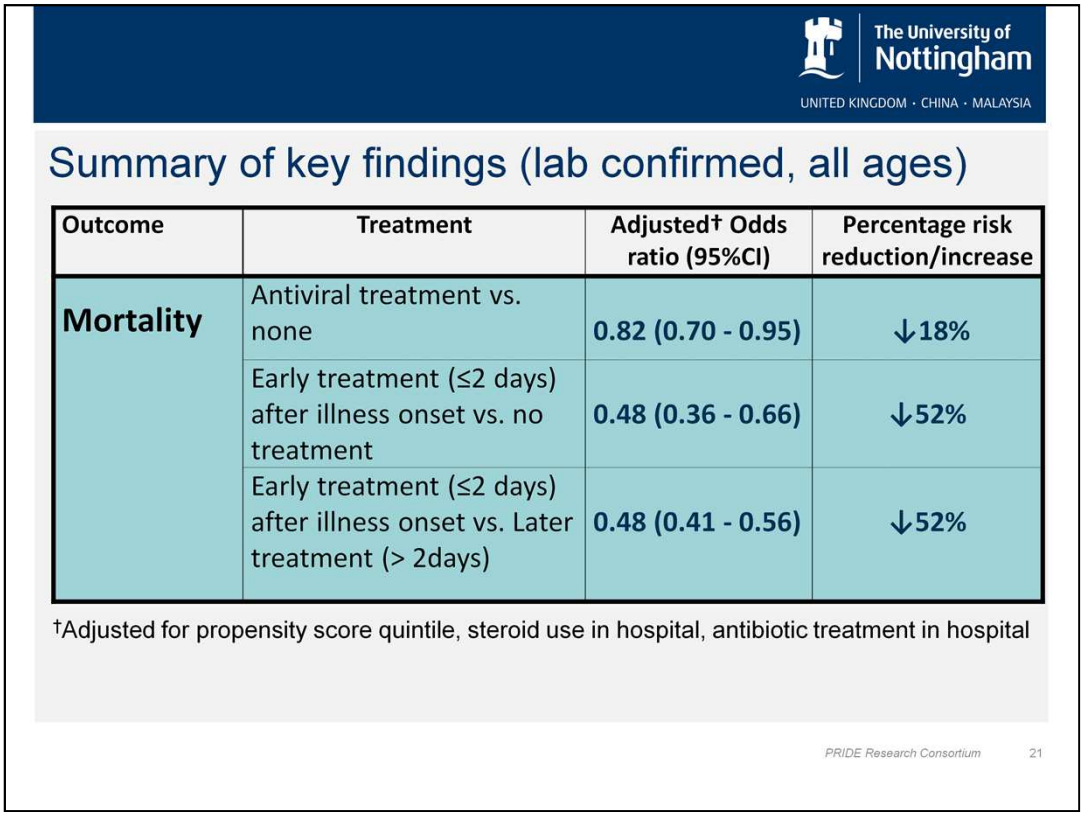
This slide shows the IPD results for ‘early NAI treatment versus no NAI treatment’.

After adjustment, the overall results for laboratory confirmed A(H1N1)pdm09 cases show a statistically significant reduction in mortality of 52%. There is a similar reduction of 50% when we consider both laboratory confirmed and clinically diagnosed cases. In adults there was a significant reduction of 62%. In pregnant women a significant reduction of 84% was seen while in critically ill patients there was a 69% significant reduction in mortality. Once again, the only sub-group for which no statistically significant reduction in mortality was seen was that comprising children.



This slide shows the IPD results for 'early NAI treatment versus late NAI treatment'.

After adjustment, the overall results for laboratory confirmed A(H1N1)pdm09 cases show a statistically significant reduction in mortality of 52%. This remains unchanged when we consider both laboratory confirmed and clinically diagnosed cases. In adults there was a significant reduction of 55%. In pregnant women a significant reduction of 73% was seen while in critically ill patients there was a 38% significant reduction in mortality. Once again, in children we do not see a statistically significant reduction in mortality.



This slide just summarises the key findings for lab confirmed cases of all ages. NAI treatment at any point was associated with a significant reduction in mortality by 18%. Early initiation of NAIs (whether compared to late treatment or no treatment) was associated with a 52% significant reduction in mortality.

A question that has been frequently asked is whether there is any point in prescribing NAIs to patients if they present more than 2 days after symptom onset. The fact that we do see a significant reduction in mortality with NAI treatment irrespective of timing of administration suggests that there is some clinical benefit in prescribing NAIs even to 'late' presenters. Subsequent slides will explore this question further.

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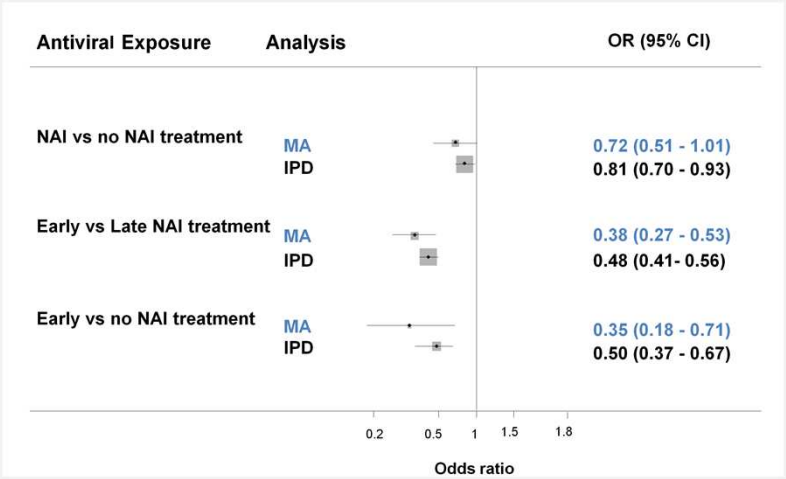
Comparison of results: meta-analysis versus IPD meta-analysis [Lab confirmed and clinically confirmed (all ages)]



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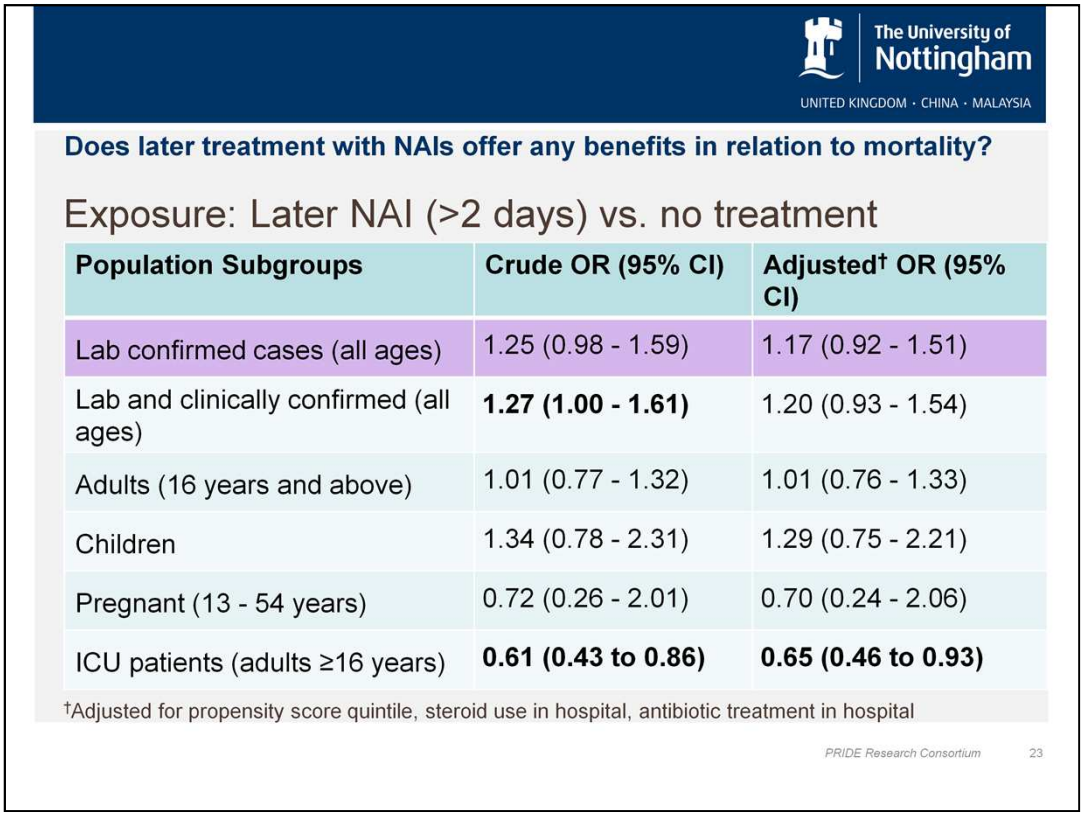
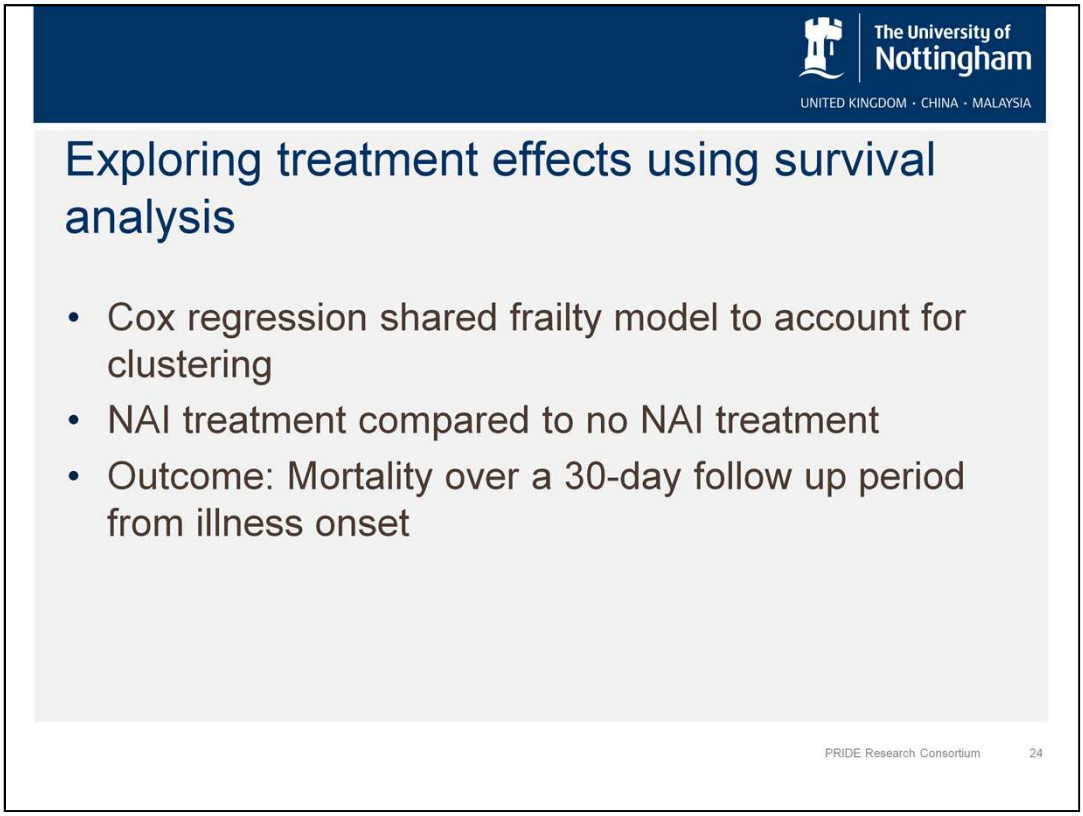
So was there any point in conducting an individual patient data meta-analysis? This slide compares the pooled results obtained from the meta-analysis of published studies (seen in blue) and those obtained from the IPD analysis (seen in black). This illustrates how the point estimates for reduction in mortality have reduced slightly with the IPD but the precision has increased i.e. we see tighter confidence intervals.



This slide explores whether treatment with NAI antivirals later than 2 days following symptom onset is of any benefit compared to no NAI treatment. We did not find a statistically significant effect between later NAI treatment and mortality except for in critically ill adult patients where even later treatment was associated with a significant reduction in mortality by 35%. This could be explained in two ways: first, the critically ill patient sub-group was more equivalent in terms of illness severity, therefore less confounded by differences in severity; this allowed us to observe a statistically significant benefit with NAI treatment. The second alternative is that viral replication is prolonged in critically ill patients so NAI antivirals have a longer time window of opportunity within which to act.



We further explored the effect of NAI antiviral treatment on mortality using survival analysis in a subset of the PRIDE study sample where exact dates of symptom onset, timing of NAI antiviral administration and mortality were known. The Cox survival analysis is generally considered a more superior statistical model to the logistic regression model and takes into account the longitudinal nature of the data. We compared NAI treatment to no NAI treatment and our outcome of interest was mortality within 30 days of symptom onset. In a survival analysis, the timing of treatment is automatically factored in which is why we did not need the prior definitions of early and late NAI antiviral use.

The Cox shared frailty model is analogous to the multilevel logistic regression model and takes into account clustering of effects by study centre. As for the multilevel logistic regression model, the survival analysis adjusted for propensity score quintile and treatment with corticosteroids and antibiotics.

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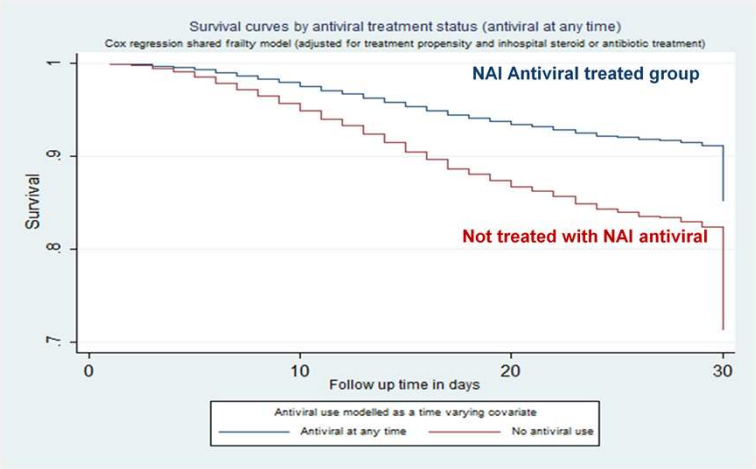
Adjusted survival curves in NAI treated and untreated patients



Decreasing survival

Adjusted Hazard Ratio: 0.51 (95% CI: 0.45- 0.58); P<0.0001

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This slide shows that those receiving NAI antiviral treatment (blue curve) were less likely to die as compared to those not receiving NAI antiviral treatment (red curve) over a 30-day follow up period. Results of the survival analysis are represented as an adjusted hazard ratio (HR) which is a conditional probability of survival at a particular time-point and in this case would be interpreted as: patients treated with NAI antivirals were about 50% less likely to die as compared to those not receiving NAI antiviral treatment at the 30-day follow-up point.

While hazard ratios and odds ratios are not directly comparable (i.e. a HR of 0.8 ≠ OR of 0.8), the message from both the logistic regression and Cox regression models is consistent- NAI antiviral treatment is significantly associated with a reduction in mortality.

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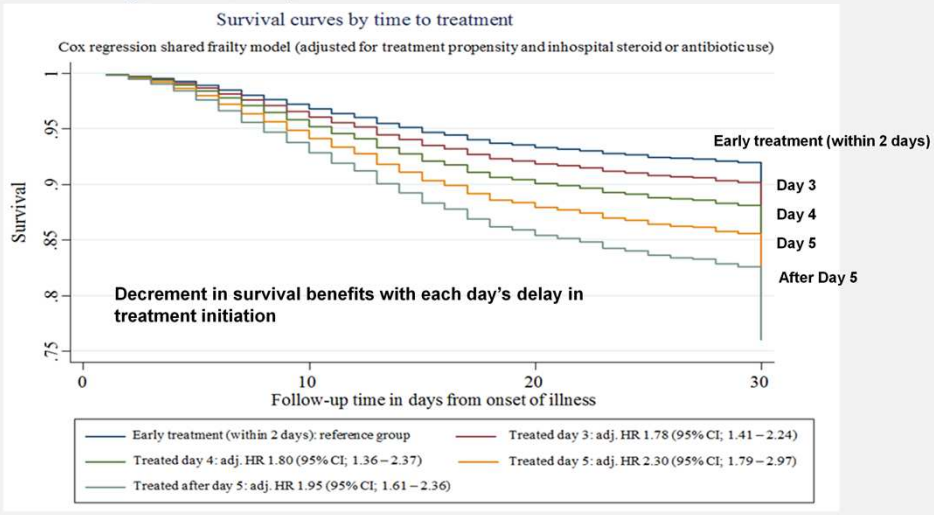
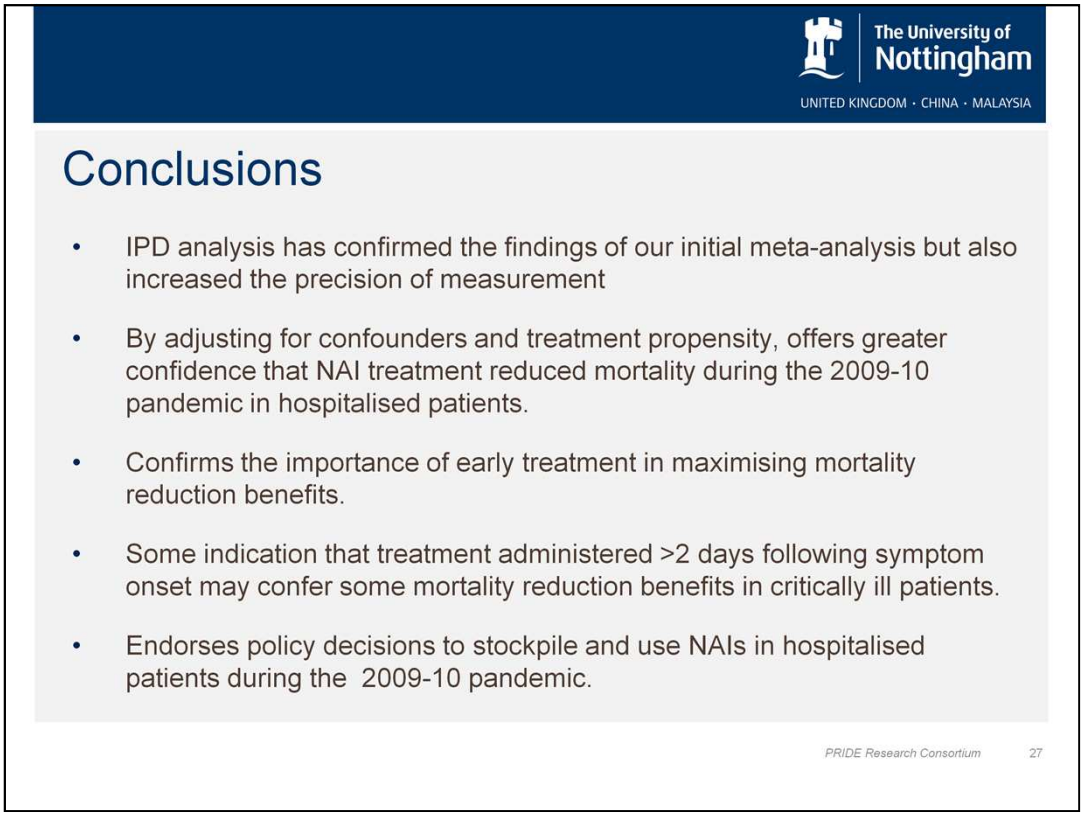
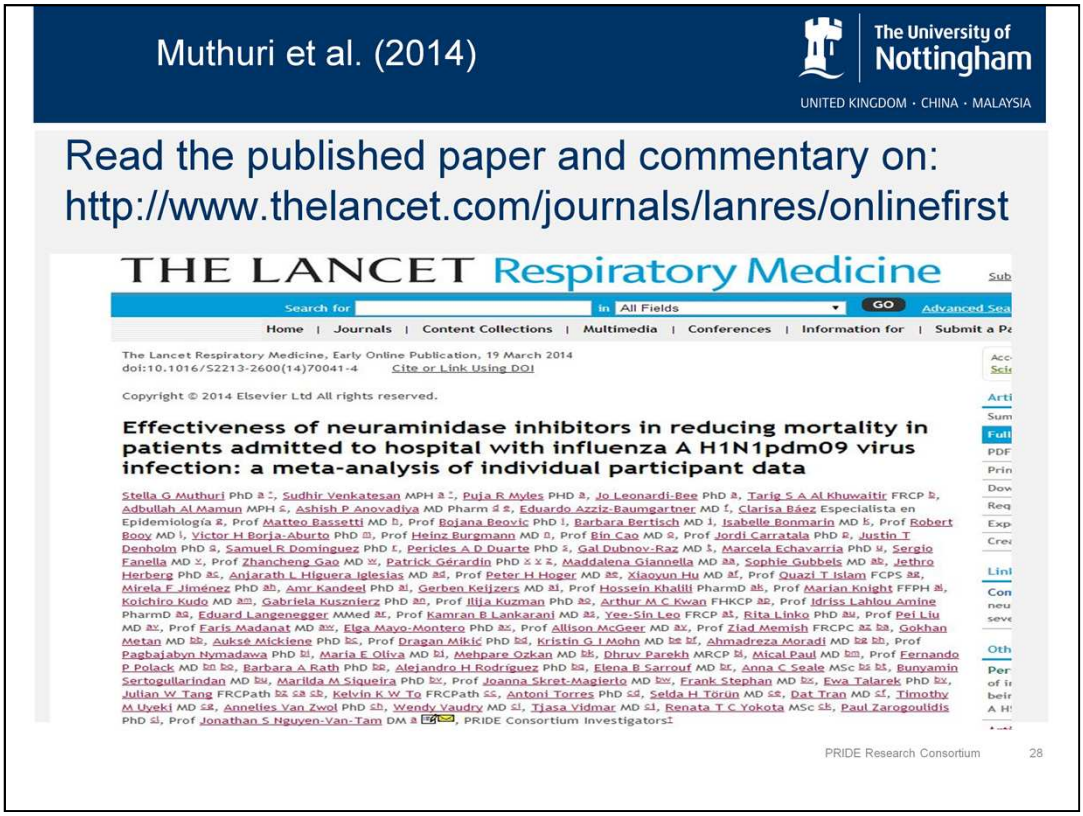
Adjusted survival curves by time to treatment initiation (**treated patients only**; n=10,791)



This slide only compares treated patients. This analysis was prompted by clinician queries about the impact of delayed treatment on potential survival benefits. This figure shows that there is a decremental survival benefit with each day's delay in treatment initiation therefore reinforcing the message that early treatment with NAI antiviral treatment has the best chance of benefiting patients.



In conclusion, the PRIDE IPD analysis has confirmed the findings of the initial meta-analysis of published studies and also increased the precision of measurement. By adjusting for confounders and treatment propensity, it offers greater confidence that NAI treatment reduced mortality during the 2009-10 pandemic in hospitalised patients. It confirms the importance of early treatment in maximising mortality reduction benefits. There is some indication that treatment administered >2 days following symptom onset may confer some mortality reduction benefits in critically ill patients. Finally, it endorses policy decisions to stockpile and use NAIs in hospitalised patients during the 2009-10 A(H1N1)pdm09 pandemic.

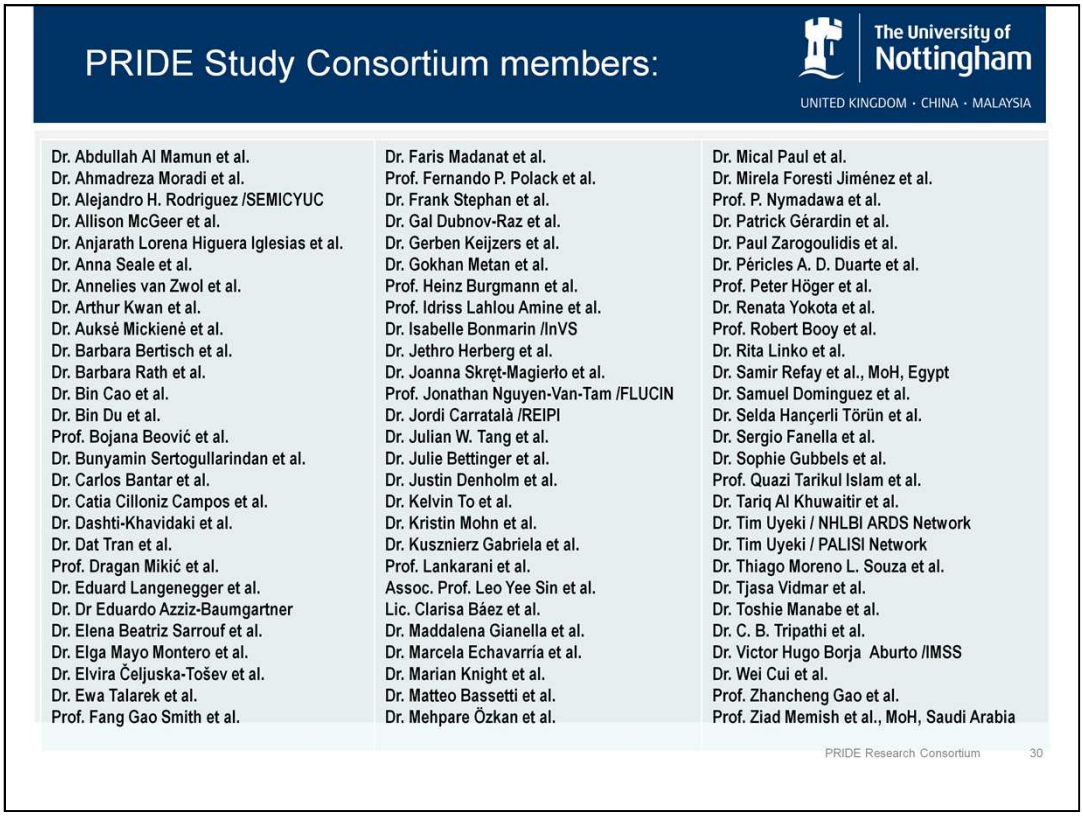


Read the published paper and commentary on:
http://www.thelancet.com/journals/lanres/article/PIIS2213-2600(14)70041-4/abstract

For a freely available pre-publication version of the full paper and the supplementary data, please refer to the PRIDE Study website:
http://www.nottingham.ac.uk/research/groups/healthprotection/projects/pride.aspx

Here you will also find the published critiques of this work as well as the authors' response along with other useful information and FAQs relating to the study.

For more information you can visit the PRIDE study website, Nottingham.



A list of the PRIDE Study Consortium members without whom this study would not have been possible.

Please insert your own contact details in this slide.

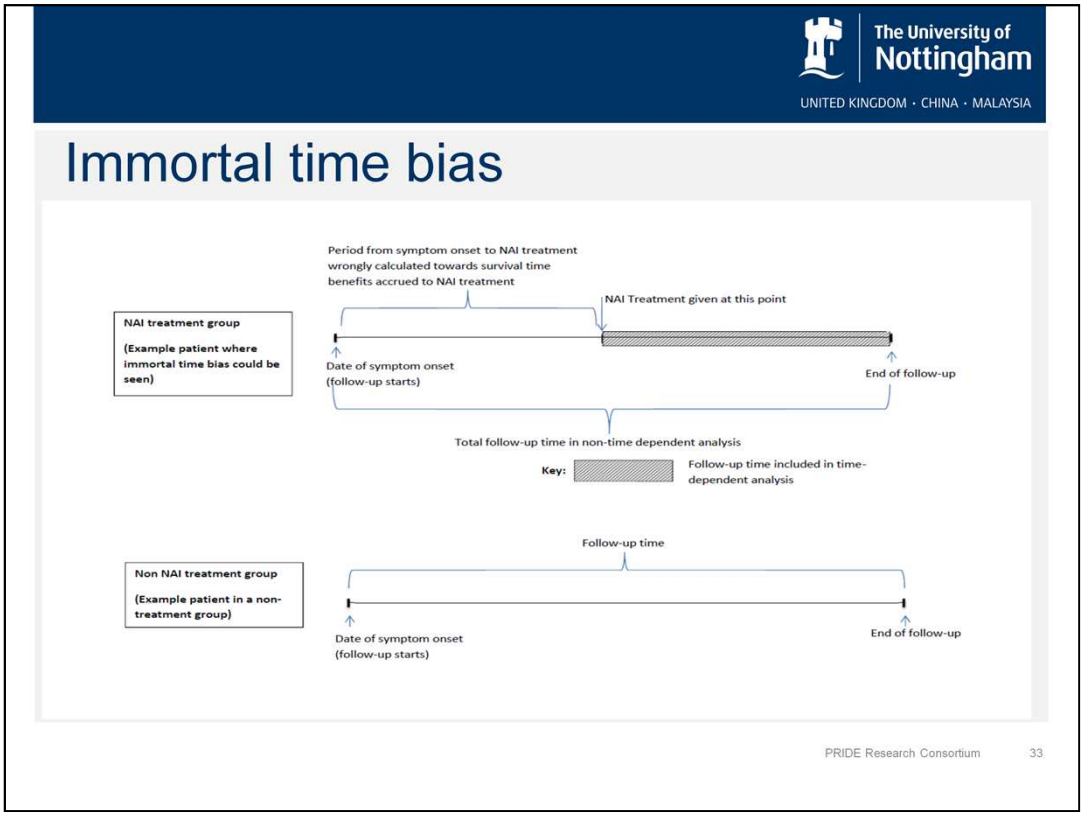
For specific questions relating to the study conduct/methodology please refer your audience to:

Dr Puja Myles (puja.myles@nottingham.ac.uk)

Professor Jonathan Nguyen-Van-Tam (jvt@nottingham.ac.uk)

For media queries and strategic collaborations/discussion, please refer to Professor Jonathan Nguyen-Van-Tam.

Useful slides in case of queries



One of the key discussions post-publication related to the survival analysis approach used in the PRIDE study. In case of queries about time-dependent biases, you may wish to use this diagram to illustrate what is meant by a time-dependent bias particularly, immortal time bias and how this can be accounted for in a survival analysis.

Time-dependent treatment effects can impact findings such that treatment can appear to be favourable as compared to no treatment because of a bias termed ‘immortal time bias’ which is observed because patients who die early do not get an opportunity to receive treatment. Therefore, when comparing treated and untreated patients, it is important to compare survival time only after treatment is initiated in the treated group (time dependent analysis). This is the approach that has been followed for the PRIDE study analysis. An alternative analysis that ignores such time-dependent effects would compare survival in both treated and untreated groups from the point of illness onset. This is explained diagrammatically in this slide.