PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effectiveness of the 13-valent pneumococcal conjugate vaccine against adult pneumonia in Italy: a case-control study in a two-year prospective cohort
AUTHORS	Prato, Rosa; Fortunato, Francesca; Cappelli, Maria; Chironna, Maria; Martinelli, Domenico

VERSION 1 – REVIEW

REVIEWER	Walter Demczuk Public Health Agency of Canada Canada
REVIEW RETURNED	01-Sep-2017

GENERAL COMMENTS	Although the effectiveness of the PCV13 vaccine to reduce disease caused by the targeted serotypes has been clearly established, a discussion of the need for such an intervention in adults is warranted. The authors recommend the implementation of PCV13 into vaccination schedules for the elderly, predicated on the lack of any observed herd immunity conferred through childhood immunization programs. They state levels of PCV13 serotypes remain high in adults despite the implementation of the childhood vaccination programs. However the time frame used in this study, upon which this statement is based, is far too short to observe herd immunity effects, which may take about 4 or 5 years to become evident. A similarly short time period between childhood PCV13 immunization and adult PCV13 serotype levels of the study is cited. The childhood pneumococcal reservoir causing adult disease has been well documented and declining rates of PCV13 serotype carriage and disease among children has clearly demonstrated herd immunity effects for adults, and the reduction of virulent strains further reduces the transmission of invasive disease. Can the authors address the question as to why PCV13 vaccination schedule in adults should be implemented when eventually the targeted PCV13 serotypes may be eradicated from the childhood reservoir? The authors should attempt to explain how the PCV13 vaccine can be effective against non-vaccine serotypes as described in this study.

REVIEWER	Sven-Arne Silfverdal Clinical sciences, Pediatrics, Umeå University, Sweden
REVIEW RETURNED	11-Sep-2017

GENERAL COMMENTS	This is a study from Italy about effectiveness of PCV13 in an adult population >65 years and as such a needed study. However, the authors need to perform a major revision in order to et it more relevant and easy to follow. Some comments. Abstract. In the abstract, the authors overestimate in the results about the vaccine effectiveness although it crosses the confidence limits, and the resuots are mostly non-significant. The introduction is a bit long and is focussing on Vaccone efficacy results from other settings, especially the US. Why not put more attention to the situation in Europe with another serotype-distribution etc. The first part of the introduction passage could be condensed. Due to possible serotype fluctuation and replacement I think it is generally more important to look at the overall vaccine effectiveness, and leave the serotype- specific one. Methods. Page 6 lines 32-46 about clinical criteria would be easier to follow in a table. I had to read it several times to really understand. The Vaccine effectiveness analysis: It seems to me that their variant of case-control method in this case overestimates the VE. Although it may in some sense control for confounding by their matching I wonder if they have data on an ecological level - before and after an implementation that could support their findings. Results. Out of 1867 eligible cases only 226 were included. Isn't it a risk for selection bias here? Or do the authors estimate this risk to be equal for cases and controls? 40 out if 226 lacked important biological data and a selection bias here can not be excluded. In table 2 it is clear that the main problem is non-significant result and and problem with a small sample size. Discussion. The discussion is unfortunately not focussed on their own findings, but on the general impact and usefulness of PCV13. Much of the text can be removed or integrated in the introduction. Please start and present your own result and discuss these. Although the result are non-significant they can stimulate to perform larger studies that would probably confirm the VE but probably with lower VE estimates than those presented here.

REVIEWER	Chris Robertson Strathclyde University Scotland
REVIEW RETURNED	18-Oct-2017

GENERAL COMMENTS	I click no for 3, 11, and 12. 3 I think that the sample is too small and there should have been a power calculation initially to give the authors an idea of how big a sample they should be recruiting. 11 and 12 was really the same reason. The author claim positive vaccine effects yet for their main endpoints they cannot claim any vaccine effect given the imprecision (very wide CIs) that they have in the data. I think that there should be a discussion of potential bias associated with the poor consent rate. All three of these points can be addressed in a revision.

	Effectiveness of the 13-valent pneumococcal conjugate vaccine against adult pneumonia in Italy: a case-control study in a two-year prospective cohort Statistical Review Chris Robertson I enjoyed reading this paper and agree that it is very important to carry out and report on these studies to provide information on vaccine effectiveness for PCV in the elderly. The statistical analysis is reasonable and confidence intervals are used appropriately. There are areas where I would have done things differently – using small sample exact procedures – and these are likely to widen the confidence intervals. It is difficult to do anything more sophisticated in view of the small sample. The main statistical weakness of the paper is the lack of a prospective power calculation. I think that the other weakness is one that the authors recognise – small numbers associated with a poor consent rate. There is a potential for this to introduce bias and it is important that he authors attempt to understand the potential biases in the data and to discuss how they might influence the conclusions. I have a good few points below and they represent things I noted as I read the paper. Some of them may have arisen because I have missed something in the paper. Page 6 Line 26. Please clarify if the 13841 patients are 65+ P6 l46-48. Please explain why these exclusion criteria were necessary. Is the discharge one part of the definition of community acquisition of pneumonia? P8 l 19-23 . could you please explain why the controls for the vaccine type effectiveness were those individuals who had pneumococcal CAP but non vaccine types. You could have used those who did not have pneumococcal CAP as well but would be measuring a different VE. You would have a bigger sample size. P8 l 28-30. Were both vaccines verified or is this just referring to PPSV23 P8 l33-43. What clinical information was collected for controls. Where they contacted concurrently with the cases. Was there any delay in getting controls, and what was the response rate? There does not appear to be any prospective power calculation. I think that his should have been done for the Ethical Review. I think that the researchers should have been able to make judgments about anticipated vaccine effects and hence provide a power for various anticipated effect sizes. P8 l 49-60. Is there any seasonality to pneumococcal CAP. If so this should be accounted for. P8 L56. In view of the small numbers I suggest calculating the CIs using exact methods, exact logistic regression for example. P9 l13. The consent rate is really low. Do you have information on who consents/does not consent? Any information on who was unable to provide a blood/sputum sample.? 35% female is strange as at the age range 65+ you would expect more women. Is this a selection bias or do men get pneumonia more than women? Table 1 – what are the p values for? What does referent mean? The sample is so low that the power of any test is low. The missing outcome data for 60 patients is a severe problem.

	P10 l26 What about the disparity between pneumoccoal and reporting where pneumococcal was more likely to be found if it was a GP source. Is this another bias? Table 2. Can you please check the numbers in relation to Table 1. There are 127 non pneumococcal CAP and 15 are vaccinated in Table 1, giving 112 unvaccinated. You have 108 – does this mean there are 4 who you do not know PCV13 status for. There are 59 pneumococcal patients and you only have 52 in the VE calculation. Are there some pneumococcal CAP patients for whom serotype is unknown? Please clarify this. I may have missed it in the text. P11, L 30-34. Exclusion of those who died might cause a small bias towards the controls being healthier. If a control is alive at the time of the case then he/she should be a potential control even if they subsequently died. I appreciate that this exclusion may be for administrative convenience of getting consent. Page 11, L 36—43. The controls were matched on age, sex and GP so they must be balanced on thee characteristics by design. I don't think it is very good to say that they differed in other characteristics without describing these differences. I know you subsequently adjust for co morbidity so is this the only characteristic. It is important to know if there were others. Also what was the consent rate among the controls.

REVIEWER	Lan Liu University of Minnesota at Twin Cities
REVIEW RETURNED	30-Oct-2017

GENERAL COMMENTS	This paper presented a study investigating the direct effectiveness of PCV13 for the prevention of adult pneumococcal CAP in Italy. Overall, the paper is clearly written. However, my main concern of this paper is how much information we could obtain from the study due to its limitations. 1. Sample size. The study identified 1867 eligible adults with only 186 is left for the CAP cohort study. The main interest of the study is to find the proof of effectiveness of the vaccine. But it seems that almost all the results have a very wide confidence interval which prevent the claim for effectiveness. For example, VE estimate was 33.3% (95% CI -93.1% to 77.0%) against pneumococcal CAP irrespective of serotype and 54.2% (95% CI -210.2% to 93.2%) against vaccine-type CAP in the cohort of adults ≥65 years. These confidence intervals are really too wide to reach an informative conclusion. 2. Study design. The study is an observational study in the sense that the treatment is assigned and then the control cases are obtained by matching on covariates. The study design limits the conclusion of the causal effect since important factors may not be matched upon. Thus, even there is a significant relationship between the vaccine and outcome observed in this study, it is possible that this is due to lack of controlling for necessary factors, making the conclusion only associational rather than causal. An ideal study design would be to randomize vaccine. However, this may or may not be ethical/feasible. But this limitation should be acknowledged in the paper.

	3. Missing data. In Table 1 footnote, it is mentioned that there are 20 patients missing in the pneumococcal group and 40 patients in the non-pneumococcal group. This is a quite high percentage of missingness since there are only 59 individuals in the pneumococcal group and 127 in the non-pneumococcal group. It is very likely that the patients are missing due to disease or death. Thus, the missingness is not at random (nonignorable). If we just make our conclusion based on the complete data, it will likely lead to misleading results. There are some minor typos in the paper. But the points mentioned above concerns me.

VERSION 1 – AUTHOR RESPONSE

Editorial Requirements:
ER.1 – Please revise the strengths and Limitations section (after the abstract) to focus on the methodological strengths and limitations of your study rather than summarizing the results.
ER.1 – OK, done.

ER.2 – Please work to improve the quality of English throughout the manuscript, either with the help of a native speaking colleague or with the assistance of a professional copyediting agency.
ER.2 – The manuscript has been edited by an English-speaking native.

Reviewer(s)' Comments to Author:

Reviewer: 1 – Walter Demczuk, Public Health Agency of Canada, Canada

Q.1.1 – They state levels of PCV13 serotypes remain high in adults despite the implementation of the childhood vaccination programs. However the time frame used in this study, upon which this statement is based, is far too short to observe herd immunity effects, which may take about 4 or 5 years to become evident. A similarly short time period between childhood PCV13 immunization and adult PCV13 serotype levels of the study is cited. The childhood pneumococcal reservoir causing adult disease has been well documented and declining rates of PCV13 serotype carriage and disease among children has clearly demonstrated herd immunity effects for adults, and the reduction of virulent strains further reduces the transmission of invasive disease. Can the authors address the question as to why PCV13 vaccination schedule in adults should be implemented when eventually the targeted PCV13 serotypes may be eradicated from the childhood reservoir?
A.1.1 – Thank you for your comments that have allowed us to discuss the most recent findings from countries where, probably, it is too short to observe PCV13 herd immunity effects. As other studies, we have concluded that the decline in pneumococcal disease incidence observed in some countries is not always be mirrored elsewhere in the same time. Even in countries with maximum herd protection achieved, studies showed there will still be residual disease that has escaped herd immunity.

Reviewer: 2 – Sven-Arne Silfverdal, Clinical sciences, Pediatrics, Umeå University, Sweden

Q.2.1 – Abstract. In the abstract, the authors overestimate in the results about the vaccine effectiveness although it crosses the confidence limits, and the results are mostly non-significant.
A.2.1 – We agree with you to be more cautious in drawing conclusions on PCV13 effectiveness from our results.

Q.2.2 – The introduction is a bit long and is focussing on Vaccine efficacy results from other settings, especially the US. Why not put more attention to the situation in Europe with another serotype-distribution etc. The first part of the introduction passage could be condensed.
A.2.2 – Thank you for the comment. We have shortened the Introduction and condensed some passages. Moreover, we have reported the most recent findings from some EU countries in the Discussion.

Q.2.3 – Due to possible serotype fluctuation and replacement I think it is generally more important to look at the overall vaccine effectiveness, and leave the serotype-specific one.
A.2.3 – We are really sorry but are not sure to have really understood what you pointed out in your comment. If you referred to the overall vaccine-type VE estimate, leaving it would be in contrast with other reviewers suggestions on how re-calculating this end point.

Q.2.4 – Methods. Page 6 Lines 32-46 about clinical criteria would be easier to follow in a table. I had to read it several times to really understand.
A.2.4 – OK, done.

Q.2.5 – The Vaccine effectiveness analysis: It seems to me that their variant of case-control method in this case overestimates the VE. Although it may in some sense control for confounding by their matching I wonder if they have data on an ecological level - before and after an implementation that could support their findings.
A.2.5 – Thank you for your comment that has allowed us to include preliminary observations of PCV13 impact on hospitalized adult pCAP in the study area.

Q.2.6 – Results. Out of 1867 eligible cases only 226 were included. Isn't it a risk for selection bias here? Or do the authors estimate this risk to be equal for cases and controls?
A.2.6 – Thank you for your observation. We have stated this risk for selection bias and have included some explanations about it.

Q.2.7 – 40 out if 226 lacked important biological data and a selection bias here can not be excluded.
A.2.7 – We have stated also this selection bias and have included some considerations.

Q.2.8 – In table 2 it is clear that the main problem is non-significant result and problem with a small sample size.
A.2.8 – OK, done.

Q.2.9 - Discussion. The discussion is unfortunately not focussed on their own findings, but on the general impact and usefulness of PCV13. Much of the text can be removed or integrated in the introduction. Please start and present your own result and discuss these. Although the results are non-significant they can stimulate to perform larger studies that would probably confirm the VE but probably with lower VE estimates than those presented here.
A.2.9 – We have acknowledged all your suggestions in the revised text.

Reviewer: 3 – Chris Robertson, Strathclyde University, Scotland (Statistical Review)

Q.3.1 – The statistical analysis is reasonable and confidence intervals are used appropriately. There are areas where I would have done things differently – using small sample exact procedures – and these are likely to widen the confidence intervals. It is difficult to do anything more sophisticated in view of the small sample. The main statistical weakness of the paper is the lack of a prospective power calculation.

A.3.1 – We agree with you. We have re-performed the statistical analysis using small sample exact procedures. Please find below the responses to each comment.

Q.3.2 – I think that the other weakness is one that the authors recognise – small numbers associated with a poor consent rate. There is a potential for this to introduce bias and it is important that the authors attempt to understand the potential biases in the data and to discuss how they might influence the conclusions.
A.3.2 –We have stated these risks for selection bias and have included some considerations.

Q.3.3 – Page 6 Line 26. Please clarify if the 13841 patients are 65+
A.3.3 – OK, done.

Q.3.4 – P6 L 46-48. Please explain why these exclusion criteria were necessary. Is the discharge one part of the definition of community acquisition of pneumonia?
A.5.4 – Yes, done.

Q.3.6 – P8 L 19-23. Could you please explain why the controls for the vaccine type effectiveness were those individuals who had pneumococcal CAP but non vaccine types. You could have used those who did not have pneumococcal CAP as well but would be measuring a different VE. You would have a bigger sample size.
A.3.6 – We have acknowledged your suggestion to re-calculate the vaccine-type VE.

Q.3.7 – P8 L 28-30. Were both vaccines verified or is this just referring to PPSV23
A.3.7 – Both vaccines, we have added this information.

Q.3.8 – P8 L33-43. What clinical information was collected for controls. Where they contacted concurrently with the cases. Was there any delay in getting controls, and what was the response rate?
A.3.8 – We have addressed these points throughout the revised text.

Q.3.9 – There does not appear to be any prospective power calculation. I think that his should have been done for the Ethical Review. I think that the researchers should have been able to make judgments about anticipated vaccine effects and hence provide a power for various anticipated effect sizes.
A.3.9 – Yes, we did statistical considerations for the Ethical Review. Overall, during the foreseen three years period of observation, we had estimated to enroll up to 750 CAP cases aged over 64 years in the selected study area. Unfortunately, the sponsor stopped the study for administrative reasons after two years. Moreover, at the beginning of the study in 2012, we had estimated a CAP sample size in the absence of a VE estimate as that then provided by the CAPITA trial.

Q.3.10 – P8 L49-60. Is there any seasonality to pneumococcal CAP. If so this should be accounted for.
A.3.10 – Sorry, we missed this information which has been now accounted for.

Q.3.11 – P8 L56. In view of the small numbers I suggest calculating the CIs using exact methods, exact logistic regression for example.
A.3.11 – OK, done.

Q.3.12 – P9 L13. The consent rate is really low. Do you have information on who consents/does not consent? Any information on who was unable to provide a blood/sputum sample?
A.3.12 – Please see A.3.2.

Q.3.13 – 35% female is strange as at the age range 65+ you would expect more women. Is this a selection bias or do men get pneumonia more than women?
A.3.13 – As observed in other studies, we had a predominance of cases in males. This gender distribution seems to follow a similar pattern in the EU countries.

Q.3.14 – Table 1 – What are the p values for? What does referent mean? The sample is so low that the power of any test is low.
A.3.14 – We agree and have deleted the p values in Table 1.

Q.3.15 – The missing outcome data for 60 patients is a severe problem.
A.3.15 – Yes, we lost several information on the last patients recruited because the study was stopped by the sponsor early.

Q.3.16 – P10 L26 What about the disparity between pneumoccoal and reporting where pneumococcal was more likely to be found if it was a GP source. Is this another bias?
A.3.16 – As we discussed about the results of the subgroup analysis of cases managed in the community, our study was designed to include the GP source in order to capture non hospitalized CAP. The high GP reporting suggests that hospital based studies may underestimate the true impact of pneumococcal disease.

Q.3.17 – Table 2. Can you please check the numbers in relation to Table 1. There are 127 non pneumococcal CAP and 15 are vaccinated in Table 1, giving 112 unvaccinated. You have 108 – does this mean there are 4 who you do not know PCV13 status for.
A.3.17 – Sorry, it was an error. We have added a footnote in Table 2.

Q.1.18 – There are 59 pneumococcal patients and you only have 52 in the VE calculation. Are there some pneumococcal CAP patients for whom serotype is unknown? Please clarify this. I may have missed it in the text.
A.3.18 – Yes, we reported that seven patients had nontypeable pneumococcal CAP in both the results and Figure 1.

Q.3.19 – P11, L30-34. Exclusion of those who died might cause a small bias towards the controls being healthier. If a control is alive at the time of the case then he/she should be a potential control even if they subsequently died. I appreciate that this exclusion may be for administrative convenience of getting consent.
A.3.19 – Yes, among the potential controls we excluded who subsequently died or left his/her GP.

Q.3.20 – Page 11, L36—43. The controls were matched on age, sex and GP so they must be balanced on the characteristics by design. I don't think it is very good to say that they differed in other characteristics without describing these differences. I know you subsequently adjust for co morbidity so is this the only characteristic. It is important to know if there were others. Also what was the consent rate among the controls.
A.3.20 – We have addressed these points throughout the revised text.

Q.3.21 – I think that the sample is too small and there should have been a power calculation initially to give the authors an idea of how big a sample they should be recruiting.
Q.3.22 – The author claim positive vaccine effects yet for their main endpoints they cannot claim any vaccine effect given the imprecision (very wide CIs) that they have in the data.
Q.3.23 – Think that there should be a discussion of potential bias associated with the poor consent rate.
All three of these points can be addressed in a revision.
A.3.21-23 – We have provided a number of considerations in the points above (i.e., A.3.9).

Reviewer: 4 – Lan Liu, University of Minnesota at Twin Cities

Q.4.1 – Sample size. The study identified 1867 eligible adults with only 186 is left for the CAP cohort study. The main interest of the study is to find the proof of effectiveness of the vaccine. But it seems that almost all the results have a very wide confidence interval which prevent the claim for effectiveness. For example, VE estimate was 33.3% (95% CI -93.1% to 77.0%) against pneumococcal CAP irrespective of serotype and 54.2% (95% CI -210.2% to 93.2%) against vaccine-type CAP in the cohort of adults ≥65 years. These confidence intervals are really too wide to reach an informative conclusion.
A.4.1 – We agree with you to be more cautious in drawing conclusions on PCV13 effectiveness from our results. However, according with other reviewer suggestions, we have re-calculated the CIs using exact methods. Another reviewer suggested to state that despite non-significant results, our study can stimulate to perform larger studies that would probably confirm the VE but probably with narrower CIs than those presented here.

Q.4.2 – Study design. The study is an observational study in the sense that the treatment is assigned and then the control cases are obtained by matching on covariates. The study design limits the conclusion of the causal effect since important factors may not be matched upon. Thus, even there is a significant relationship between the vaccine and outcome observed in this study, it is possible that this is due to lack of controlling for necessary factors, making the conclusion only associational rather than causal. An ideal study design would be to randomize vaccine. However, this may or may not be ethical/feasible. But this limitation should be acknowledged in the paper.
A.4.2 – We are really sorry but cannot agree with you. We think that the limitation you pointed out does not pertain to the aim of a study as that we designed and performed. We clearly stated that this was a post-licensure real-world study evaluating the direct impact of an ongoing adult PCV13 vaccination programme.

Q.4.3 – Missing data. In Table 1 footnote, it is mentioned that there are 20 patients missing in the pneumococcal group and 40 patients in the non-pneumococcal group. This is a quite high percentage of missingness since there are only 59 individuals in the pneumococcal group and 127 in the non-pneumococcal group. It is very likely that the patients are missing due to disease or death. Thus, the missingness is not at random (nonignorable). If we just make our conclusion based on the complete data, it will likely lead to misleading results.
A.4.3 – Yes, we lost several information on the last patients recruited because the three years study foreseen was stopped by the sponsor for administrative reasons after two years. Although the missing outcome data for 60 patients is a problem, this did not directly influence either the primary or secondary end points estimates.

Q.4.4 – There are some minor typos in the paper.
A.4.4 – Thank you, we have revised the manuscript.

VERSION 2 – REVIEW

REVIEWER	Chris Robertson Strathclyde University Scotland UK
REVIEW RETURNED	18-Jan-2018

GENERAL COMMENTS	The authors have addressed all my comments in a reasonable

	fashion. Unfortunately there are aspects of the manuscript which are still a bit weak. This is not a fault of the analysis but is really just a reflection of the impact of stopping the study early and having a much smaller recruitment than originally planned. I note your response to the power analysis. This is not included in the manuscript and I think that this should be highlighted together with the target sample size as it explains why the study is small.

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 3 – Chris Robertson, Strathclyde University, Scotland (Statistical Review)

Q.3.1 – The authors have addressed all my comments in a reasonable fashion. Unfortunately there are aspects of the manuscript which are still a bit weak. This is not a fault of the analysis but is really just a reflection of the impact of stopping the study early and having a much smaller recruitment than originally planned. I note your response to the power analysis. This is not included in the manuscript and I think that this should be highlighted together with the target sample size as it explains why the study is small.

A.3.1 – We have acknowledged your good suggestion in the final version of the paper, thank you.

VERSION 3 – REVIEW

REVIEWER	Chris Robertson Strathclyde University Scotland
REVIEW RETURNED	15-Feb-2018

GENERAL COMMENTS	The points raised in the previous review have been covered