Author’s response to reviews

Title: Increased the risk of pulmonary hypertension following premature birth

Authors:
Estelle Naumburg (estelle.naumburg@umu.se)
Lars Söderström (lars.soderstrom@regionjh.se)

Version: 1 Date: 12 Mar 2019

Author’s response to reviews:

BPED-D-18-00685

Increased the risk of pulmonary hypertension following premature birth

Estelle Naumburg, PhD.; Lars Söderström

BMC Pediatrics

Dear Editor,

Thank you for the careful revision of our manuscript. We have now revised the manuscript "Increased the risk of pulmonary hypertension following premature birth" (BPED-D-18-00685) according to reviewers suggestions, please see our reply. The manuscript has also been revised by a language editor.

The previous unacceptable degree of overlap in text covered a part where a description of study-population which was previously published by our group. This has now been rewritten and a full description is referred to.

Best wishes,

Estelle Naumburg, MD PhD

Umeå University
Reply to reviewers.

Reviewer reports:

Michael Nyp (Reviewer 1): Thank for the opportunity to review the manuscript entitled "Increased the risk of pulmonary hypertension following premature birth" currently under consideration for publication in BMC Pediatrics. In this manuscript the authors report on a population-based registry study for risks of PAH. The authors' findings are interesting showing premature infants had a higher risk of PAH at greater than 5 years of age and is more frequent over the last few years. Although the authors' observations are interesting there remains some unresolved issues with the manuscript which are outlined below.

1) The overall structure of the study is difficult to follow and may not be the best approach for the aim of the study. The presumption in the aim states PAH risk following premature birth but not all the controls were premature. The design of the study for the stated aim do not line with study design. I would suggest changing the aims or modifying the methods to align with aim better.

Reply: Thank you for your comment concerning the study design and aim of the study. This is a case-control-study, which means that all cases where patients with PAH and controls were children and adults without PAH. It is a common design for rare diseases, such as PAH. Premature birth was one of several risk factors assessed, and can thus be found in cases as well as controls. By this study design we calculated the adjusted risk factor, calculating the impact on other known risk factors such as congenital heart disease, gender, chromosomal abnormalities, congenital diaphragm hernia and acute lung disease and premature birth, for PAH. To clarify this we rephrased the aim of the study, Abstract: background page 2 and Method section, first paragraph, page 4.

2) Abstract is overall written well but again study design makes the study difficult to follow. Controls matching to birth year and delivery may not be the best control model. The lack of details about factors included in the logistic regressions make the result difficult to interpret. Since the aim of the study is stated as assessing risks of PAH following premature birth then PAH associated with premature birth cannot be a result.

Reply: This is a national-based study, and to rule out the risk of risk of selection bias by differences in medical neonatal and pediatric care and survival rates. We believe this is the best matching. We assessed factors associated with pulmonary hypertension in children and adults. PPHN (persitant pulmonary hypertension) is a neonatal condition and was assessed among other risk factors. We believe that PPHN and PAH are two different diseases, where PPHN is part of
the fetal circulation, whereas PAH has another origin. Maternal hypertension, congenital diaphragm herniation, congenital heart defects, chromosomal abnormalities, PPHN and female sex were all risk factors independently associated with PAH, even after adjustments were made. Premature birth was adjusted to each of the above described risk factors, but was still an independent risk factor for PAH.

3) The background section lack sufficient details to get to why this study was done.

Reply: We have in two previous studies found an increased risk for PAH following premature birth, but these studies were performed before and after the introduction of surfactant and antenatal corticosteroid treatments was in clinical use in Sweden. The risk increased between these two studies (OR=3.08 (95%CI; 1.21 – 7.87) vs. OR=8.46 (95% CI 2.97-24.10)). The aim of this study was to make risk estimations over time in order to assess the impact of the improved ante- and neonatal care. This has been described in more detail in the background section, last sentence.

4) The methods lack important details including why the study population was study, certainly treatment and care of neonates has changes a lot since the 1970’s make the overall study population difficult to assess significance of their finding and focusing more on the more recent data or even comparing early epoch to later epoch to assess for risk of PAH related to premature birth maybe a different study design. There is a lack of details in the logical regression design and what known confounding factors were controlled for.

Reply: We agree with the reviewer that great improvements of neonatal care has occurred since the 1970’s. The survival rates have improved dramatically and we now expect almost 100% survival in Sweden, even in extreme premature births. Our hypothesis is that factors in relation to premature birth, yet unknown, may play an important role in the development of PAH in childhood and among adults. The best way to assess this hypothesis was to make a study covering a long time-period when great improvement were made in neonatal care.

The aim of this study was to make risk estimations over time in order to assess the impact of the improved ante- and neonatal care. This has been described in more detail in the background section, last sentence.

5) Results are hard to follow with poor quality tables and figures. Data is really hard to interpret. Recommend more details in methods section and clarifying the tables and figures. Improving methods and study design with make results easier to interpret.
Reply: Thank you for these suggestions! We have now included more details in the method section, especially with regard to exposure data. Tables 1 and 2 have already with much details, and we have tried to improve the quality by reorganizing the tables and marked the significant results. According to the reviewers suggestions we have now clarified the result section with more details and sub-headings.

6) Due to the difficulty listed in the methods and results section, the discussion section is difficult to follow. Some areas of the discussion lack important details (known risk factors, prematurity survival in the 70's, discrepancies between prematurity survival in the 70's and neonatal deaths of only 8% in 1973)

Reply: Yes, we certainly agree with the reviewer that the survival rates have improved over the years. This is discussed in the whole second paragraph of the discussion, and clarified further by this sentence: “The risk of development of pulmonary hypertension for a child born premature during the 1970’s and 1980’s and who reached adulthood, must be regarded as less likely than today, mainly because children did not survive the neonatal period during these years”.

7) The conclusion is not clear on which new factors have the authors identified that may play a role in risk of PAH developing in preterm infants.

Reply: This a national-based case-control study test the hypothesis of increased risk of PAH in children and adults following premature birth, at the same time improved neonatal care and raised survival rates are seen. We discuss if yet unknown factors, such as pulmonary vascular growth, may have an impact on these risk. However, these factors have to be studied by other research methods. The conclusion have been further stressed in this revised version.

Reviewer 2 (Reviewer 2): PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?

Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?
Yes - the approach is appropriate

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?
Yes - experiments and analyses were performed appropriately

Statistics - Is the use of statistics in the manuscript appropriate?
Yes - appropriate statistical analyses have been used in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?
No - there are minor issues

OVERALL MANUSCRIPT POTENTIAL - Is the current version of this work technically sound? If not, can revisions be made to make the work technically sound?
Probably - with minor revisions

PEER REVIEWER COMMENTS:
GENERAL COMMENTS: This study seems to be quite interesting. The study is more valid as it is population-based. The discussion however needs more information.

REQUESTED REVISIONS:
This study seems to be quite interesting. In the abstract please minimize the methods component and add more useful results.

Reply: Thank you for your suggestions regarding the abstract. The method section has been reduced and added more results.
Methods are mostly well explained. You must include on what grounds the PAH was diagnosed in these cases.

Reply: Thank you! The basis for PAH-diagnosis has now been included in the method section, and described in the section “study-population”.

In results, over 26 years, there were only 128 cases of pulmonary hypertension? Is this number correct? Among the cases, 10 had chromosomal abnormalities, if you exclude this and analyze further what is the risk of PAH vs preterm-please provide statistics.

Reply: Yes, this number (128 cases) is correct. As you see in Table 1, fewer cases were born during the 1980’s and 1990’s. We believe few premature children survived during the 1980’s and 1990’s, and thus only a few children and adults were able to develop PAH and could be included among the cases.

In all analysis we adjusted for other confounding factors, such as chromosomal abnormalities. We believe that the impact on PAH by chromosomal abnormalities, can influence the risk of PAH as well as premature birth. By adjusting for chromosomal abnormalities in our study, we rule out this impact. According to your request, we have made new analyzes (please see below):
1) excluding the variable chromosomal abnormalities: the independent association between prematurity and risk for PAH was OR=3.57 (95% CI 1.74-7.35) and by 2) excluding children with chromosomal abnormalities, the independent association between prematurity and risk for PAH was OR=4.32 (95% CI 2.02-9.21). This has been discussed in the discussion section, page 9, fourth paragraph.

Original analysis

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>OR</th>
<th>p</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>128</td>
<td>768</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premature</td>
<td>37</td>
<td>47</td>
<td>4.48</td>
<td>0.0001</td>
<td>2.10</td>
<td>9.52</td>
</tr>
<tr>
<td>Kon</td>
<td>72</td>
<td>346</td>
<td>1.69</td>
<td>0.0277</td>
<td>1.06</td>
<td>2.71</td>
</tr>
<tr>
<td>SGA</td>
<td>7</td>
<td>13</td>
<td>4.06</td>
<td>0.0310</td>
<td>1.14</td>
<td>14.49</td>
</tr>
<tr>
<td>LGA</td>
<td>6</td>
<td>21</td>
<td>3.53</td>
<td>0.0212</td>
<td>1.21</td>
<td>10.31</td>
</tr>
<tr>
<td>Variable</td>
<td>Cases</td>
<td>Controls</td>
<td>OR</td>
<td>p</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>------------</td>
<td>-------</td>
<td>----------</td>
<td>-----</td>
<td>---------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
<td>768</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premature</td>
<td>37</td>
<td>47</td>
<td>3.57</td>
<td>0.0005</td>
<td>1.74</td>
<td>7.35</td>
</tr>
<tr>
<td>Kon</td>
<td>72</td>
<td>346</td>
<td>1.79</td>
<td>0.0129</td>
<td>1.13</td>
<td>2.85</td>
</tr>
<tr>
<td>SGA</td>
<td>7</td>
<td>13</td>
<td>3.45</td>
<td>0.0451</td>
<td>1.03</td>
<td>11.60</td>
</tr>
<tr>
<td>LGA</td>
<td>6</td>
<td>21</td>
<td>2.96</td>
<td>0.0377</td>
<td>1.06</td>
<td>8.25</td>
</tr>
<tr>
<td>CPD</td>
<td>15</td>
<td>3</td>
<td>13.38</td>
<td>0.0036</td>
<td>2.34</td>
<td>76.56</td>
</tr>
<tr>
<td>PPHNPDA</td>
<td>27</td>
<td>10</td>
<td>14.36</td>
<td>0.0000</td>
<td>5.33</td>
<td>38.70</td>
</tr>
<tr>
<td>CHD</td>
<td>23</td>
<td>10</td>
<td>16.46</td>
<td>0.0000</td>
<td>6.76</td>
<td>40.07</td>
</tr>
<tr>
<td>M_HT</td>
<td>15</td>
<td>37</td>
<td>2.51</td>
<td>0.0337</td>
<td>1.07</td>
<td>5.89</td>
</tr>
</tbody>
</table>

All observations with chromosomal = 1 removed

<table>
<thead>
<tr>
<th>Cases</th>
<th>Controls</th>
<th>OR</th>
<th>p</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>118</td>
<td>767</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premature</td>
<td>35</td>
<td>47</td>
<td>4.32</td>
<td>0.0002</td>
<td>2.02</td>
</tr>
</tbody>
</table>
Discussion: Please address the limitations of this study. Please also inform about the factors that are not explored in this study. I believe that large number of preterm birth did not have pulmonary hypertension in your group (considering the 6% as preterm birth)-this factor is not discussed. Hence it is likely that factor other than preterm birth is contributing more to pulmonary hypertension. Please inform also below what gestational age neonates are at increased risk for PAH.

Reply: Thank you for this suggestion. We have addressed limitation of the study in the discussion section. As in most case-controls studies, exposures to the assessed risk factors is most often present in both groups. However, the exposure was much more common among cases and thus the risk association was significant. This has been discussed in more detail in the discussion section, third paragraph.

Conclusion: Modify, Preterm birth along with other factors significantly contribute to PAH.

Reply: Thank you for this suggestion to improve the conclusion!

ADDITIONAL REQUESTS/SUGGESTIONS:

Minor language polishing

Reply: Thank you for your suggestion about language improvements. The manuscript has been reviewed by a language editor.
Note: This reviewer report can be downloaded - see attached pdf file.

If improvements to the English language within your manuscript have been requested, you should have your manuscript reviewed by someone who is fluent in English. If you would like professional help in revising this manuscript, you can use any reputable English language editing service. We can recommend our affiliates Nature Research Editing Service (http://bit.ly/NRES_BS) and American Journal Experts (http://bit.ly/AJE_BS) for help with English usage. Please note that use of an editing service is neither a requirement nor a guarantee of publication. Free assistance is available from our English language tutorial (https://www.springer.com/gb/authors-editors/authorandreviewertutorials/writinginenglish) and our Writing resources (http://www.biomedcentral.com/getpublished/writing-resources). These cover common mistakes that occur when writing in English.

Reply: The manuscript has been reviewed by a language editor.