Author's response to reviews

Title: Early life microbial exposure and fractional exhaled nitric oxide in school-age children: a prospective birth cohort study.

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Author's response to reviews: see over
Reviewer's report:

I am satisfied that the authors have addressed most of the issues I raised, although I still remain concerned about 2 of them: FeNO as a marker of eosinophilic inflammation and the relationship with allergic sensitisation. These were addressed but not satisfactorily. I consider these both important issues but one of them, inflammation, I will categorise as a discretionary revision. This is because there is a general acceptance that FeNO is a marker of eosinophilic airway inflammation (despite ongoing uncertainty).

Major compulsory revision

The authors were able to investigate an objective marker of allergic sensitization (IgE) that had been measured in some, but not all, of the children. However, the inclusion of allergic sensitisation did attenuate the effect sizes such that they lost significance, although as the authors stated the direction of the effects did not change.

Early exposure to dogs and endotoxin have been associated with reduced allergic sensitisation in later childhood. Allergic sensitisation, with and without disease, has been associated with increased FeNO. Therefore, it is possible that in this study FeNO was a marker of allergic sensitisation and the early exposures are associated with sensitisation and not inflammation per se. Did sensitization remain significant when included in the models? I am aware that sensitization was measured at different time points for each of the centres and I agree that this adds a level of complexity. However, no attempt was made to explore the potential implications of these relationships.

We appreciate your comment. We have performed additional sensitivity analyses in order to disentangle the relationships between FeNO, sensitization and the mentioned exposure variables (early life endotoxin concentration and dog ownership).

Allergic sensitization (sIgE≥0.35 kU/l) was not statistically significantly associated with endotoxin concentrations during early life and dog ownership in the first 2 years of life in none of the cohorts (see Table 1 in this document). Therefore, we do not have an indication that in our study population the early exposures are associated with sensitization and not inflammation (FeNO) per se.

In this regard, we have added the following sentences in the discussion section (p17-18):

“In addition, we considered the possibility that the early life exposures evaluated in our study were associated with FeNO through an association with atopy and not through airway inflammation. The statistical analyses with atopy as outcome did not show significant associations between atopy and endotoxin concentrations or early life dog ownership (data not shown).”

Regarding the association between FeNO and sensitization, we have re-run the models excluding the exposure variables. Table 2 in this document shows the
coefficients for sensitization not adjusted for endotoxin or dog ownership (Model 1), adjusted for endotoxin (Model 2) and adjusted for dog ownership (Model 3). Also, we have run the models including interaction terms between sensitization and endotoxin, and sensitization and dog ownership. The results are also shown in Table 2 (Models 4 and 5).

The differences in the coefficients for sensitization across the three models are minimal in the three cohorts, and the coefficients remain statistically significant after inclusion of the exposure variables (endotoxin or dog ownership) in the models. In addition, the coefficients for the interaction terms (sIgE*endotoxin or IgE*dog ownership) were not statistically significant (p-value>0.1).

These results suggest that the relationship between FeNO and sensitization in the study population is not modified by the concentration of endotoxin during early life or by dog ownership. The results form Table 2 were already mentioned in the manuscript (p20 of the discussion section).

Tables:

Table 1. Adjusted associations (OR) between allergic sensitization and early life exposures.

<table>
<thead>
<tr>
<th></th>
<th>LISA OR</th>
<th>p-value</th>
<th>PIAMA OR</th>
<th>p-value</th>
<th>INMA OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>log - endotoxin</td>
<td>1.07</td>
<td>0.773</td>
<td>1.11</td>
<td>0.595</td>
<td>1.01</td>
<td>0.936</td>
</tr>
<tr>
<td>Dog ownership in the first 2 years of life</td>
<td>0.28</td>
<td>0.263</td>
<td>1.48</td>
<td>0.512</td>
<td>0.68</td>
<td>0.415</td>
</tr>
</tbody>
</table>

Adjusted for sex, parental education and asthma. The model including log transformed endotoxin concentrations is also adjusted for season of dust sampling.

Table 2. Adjusted associations (β coefficients) between FeNO and allergic sensitization (sIgE≥0.35 kU/l), and β coefficients for the interaction terms between sIgE and endotoxin, and sIgE and dog ownership.

<table>
<thead>
<tr>
<th>Sensitization (sIgE≥0.35 kU/l)</th>
<th>LISA β</th>
<th>p-value</th>
<th>PIAMA β</th>
<th>p-value</th>
<th>INMA β</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1 (no endotoxin/dog ownership)</td>
<td>0.45</td>
<td>&lt;0.001</td>
<td>0.47</td>
<td>&lt;0.001</td>
<td>1.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 2 (including endotoxin)</td>
<td>0.50</td>
<td>&lt;0.001</td>
<td>0.47</td>
<td>&lt;0.001</td>
<td>1.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 3 (including dog ownership)</td>
<td>0.41</td>
<td>0.001</td>
<td>0.46</td>
<td>&lt;0.001</td>
<td>1.32</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Interaction terms

<table>
<thead>
<tr>
<th>Sensitization * dog ownership (&lt;2 years old)</th>
<th>LISA β</th>
<th>p-value</th>
<th>PIAMA β</th>
<th>p-value</th>
<th>INMA β</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 4 (interaction endotoxin)</td>
<td>0.06</td>
<td>0.575</td>
<td>-0.11</td>
<td>0.287</td>
<td>-0.02</td>
<td>0.822</td>
</tr>
<tr>
<td>Model 5 (interaction dog ownership)</td>
<td>-0.73</td>
<td>0.280</td>
<td>-0.42</td>
<td>0.190</td>
<td>-0.06</td>
<td>0.843</td>
</tr>
<tr>
<td>sensitization * dog ownership (≥2 years old)</td>
<td>-0.48</td>
<td>0.262</td>
<td>-0.22</td>
<td>0.600</td>
<td>-0.18</td>
<td>0.695</td>
</tr>
</tbody>
</table>

Model 1: adjusted for sex, age of FeNO measurement, asthma, parental smoking at the moment of the FeNO measurement, and parental education.
Model 2: adjusted for sex, age of FeNO measurement, asthma, parental smoking at the moment of the FeNO measurement, parental education, season of dust sampling and endotoxin concentrations.
Model 3: adjusted for sex, age of FeNO measurement, asthma, parental smoking at the moment of the FeNO measurement, parental education and dog ownership.
Model 4: adjusted for sex, age of FeNO measurement, asthma, sensitization, parental smoking at the moment of the FeNO measurement, parental education, season of dust sampling and endotoxin concentrations.
Model 5: adjusted for sex, age of FeNO measurement, asthma, sensitization, parental smoking at the moment of the FeNO measurement, parental education and dog ownership.
Discretionary revision

The authors have acknowledged there is some uncertainty around what FeNO represents but state that it is ‘..most extensively studied biomarker of airway inflammation and its non-invasive nature makes it suitable for epidemiological studies’ (p.17). They continue to explicitly state on a number of occasions (abstracts, Introduction p5, Discussion p16, 18) they that they are measuring FeNO as a biomarker of eosinophilic airway inflammation. I would argue that just because it is the most extensively studied biomarker of inflammation doesn’t necessarily make it a biomarker of inflammation. In the extensive literature around FeNO there is evidence that brings the relationship between FeNO and eosinophilic inflammation into question (eg. it doesn’t exist in the absence of atopy, it varies depending on the biological compartment where eosinophils are measured, the relationship changes with treatment in a discordant manner). By no means do I discard the possibility that FeNO reflects some aspect of (allergic) airway inflammation but I still feel there is sufficient uncertainty to warrant more cautious language.

We have modified the mentioned sections in order to be more cautious with the statement that FeNO is a marker of airway inflammation.