Reviewer's report

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

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Reviewer: Peter Franklin

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Overall comments

In this study the authors have investigated early life exposures (mould and pets) on exhaled nitric oxide (FeNO) in childhood. The paper is well written and the design, in the main, is sound. The analyses seem appropriate and the authors have mostly interpreted the results adequately. However, I have some concerns over the use of FeNO as a single outcome measure for the purpose of the study as well as the lack of inclusion in the analyses of some important factors that can affect FeNO, or at least the lack of acknowledgement of the potential importance of these factors.

Many studies have investigated the associations between environmental exposures and FeNO but none, that I am aware of, have investigated this so distant from the exposure (8 – 10 years). This makes the idea novel but given the number of factors that can influence FeNO in both the short and long term it is harder to justify the observed associations. This is particularly so as I would suggest the understanding of what FeNO represents, particularly in the general (ie those not necessarily with resp disease) population is still uncertain (see below). The time factor does not make the concept wrong and the authors have tried to account for some of the factors that could also affect FeNO, including asthma and allergy. However, some important factors have not been assessed or acknowledged: at least it is not clear if they have or not. These include, but are not restricted to, atopic sensitization (independent of allergic disease), current exposures, season of testing and current medications. Some of these data may be available

For this study FeNO is being measured as a non-invasive biomarker of eosinophilic inflammation (implied in last paragraph of Introduction and stated explicitly in the 1st paragraph of Discussion). It is very much an indirect marker and a number of uncertainties about FeNO, NO biology and eosinophilic airway inflammation remain. Although there is reasonably, but not totally, consistent evidence of a moderate correlation between FeNO and eosinophils, what FeNO truly reflects is still not known (this is nicely summed up by Teague JACI 2010; 125: 1234). Indeed some of the responses of FeNO to environmental exposures are not always consistent with the eosinophilic inflammation, with which FeNO is most closely related. The authors do focus on inflammation throughout the text but correctly imply that both the biological and clinical implications of their
findings, if any, are not known

Major Compulsory Revisions

1. Important data that can affect FeNO are either missing or not included in the analyses. If they are available they should be assessed and if not they should be acknowledged. I am aware that there are potentially many things that may have some effect on FeNO and I am not suggesting that everything that has been published needs to be considered. The authors state that ‘Potential confounders were a-priori identified from the literature and selected based on their relationship with FeNO and the exposure variables in the present study.’ (2nd Para, Statistical analyses) so maybe some of the factors I mention below were indeed tested. If so I would suggest a full list of variables that were considered and tested should be included in the supplementary material. Important factors that I was unclear about and feel should have been included in the analyses include;

• An objective measure of atopic sensitization: The authors have taken into account reported allergy but atopy per se may also be important. It is possible that many of the non-allergic children are SPT positive (atopic). Indeed it could be up to 25%.

• Current exposures: There are many studies of current environmental exposures and FeNO, some which were referenced in the Introduction. These include, among other things, air pollution and current allergen exposure. Different air pollutants have been associated with increased FeNO while current allergen exposure has been shown to increase FeNO in sensitised children (these are referenced in the manuscript - ref 34, 35, 37). The only ‘current’ exposures mentioned in the manuscript were location of home and parental smoking (it is not clear if it is current smoking or ever smoking). Even pets are classified as none, ever in 1st 2 years, ever after 1st 2 years but there is no measure of pet ownership at time of FeNO test.

• Season: This may be particularly important for those who had seasonal rhinitis for the reasons stated above (relationship between sensitization and exposure)

• Medications: Data on asthma medication was available and used to define asthmatics but it is not clear if they were also included in analyses. What about treatment for rhinitics (nasal steroids)?

2. The differences in FeNO for the different levels of exposure seemed to be small and were mostly of borderline significance. It is possible that these were purely statistical results with no biological or clinical relevance. This possibility is not raised

Discretionary Revisions

1. I suggest more caution is used when discussing FeNO as a biomarker of eosinophilic inflammation and some of the uncertainty around this be acknowledged
2. In the 3rd paragraph of the Discussion the authors state ‘FeNO is one of the few tests that has diagnostic value in asthma.’. I don’t agree and even the ATS guideline that is referenced has acknowledged that their recommendation that ‘FeNO may be used to support the diagnosis of asthma’ is a weak recommendation based only on moderate quality of evidence

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests