Author's response to reviews

Title: Exhaled nitric oxide and airway hyperresponsiveness in workers: a preliminary study in lifeguards

Authors:

Valérie Demange (valerie.demange@inrs.fr)
Abraham Bohadana (abraham.bohadana@nancy.inserm.fr)
Nicole Massin (nicole.massin@inrs.fr)
Pascal Wild (pascal.wild@inrs.fr)

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Author's response to reviews: see over
Dear Editor,

Please find below a cover letter giving a point-by-point response to the reviewers comments.

Reviewer: Lidwien A Smit

This paper describes a pilot study showing a correlation between FENO and AHR in 44 swimming pool attendants. The topic is interesting, but the preliminary character and the low number of subjects limit interpretation and adequate investigation of potential confounding factors.

We agree with Dr Smit’s statement as to a limited interpretation of our findings.

The low number of subjects: it is a pilot study, so the sample size has not to be large enough to answer a research question and it strengthens the results to be found in a limited population.

Adequate investigation of potential confounding factors: we use predicted values of FENO to get rid of confounding factors and we analyse the relation between AHR and FENO taking into account personal history of clinical markers of atopy, gender, smoking and age (as mentioned in the paragraph “Results”).

Major compulsory revisions

1. The study describes a preliminary study, but the rationale of undertaking a pilot study in only few subjects is not explained.

Our team has a long experience of the use of the methacholine bronchial challenge (MBC) test in occupational settings, since the early nineties. The aim of this preliminary study was to study the
possibility to supplement this MBC test with the FENO measurements in respiratory epidemiological studies at the workplace. As mentioned in the paper, we chose an occupational population which had previously been shown to have a high prevalence of AHR.

We have included this rationale in the paper at the end of the introduction.

2. The authors use predicted values derived from a population-based study in New Zealand (Travers et al.). There is another, larger study in Sweden that has published other reference equations (Olin et al, Chest 2006). The choice of reference values should be motivated, as there is no standard way to define FENO reference values. The FENO measurements in the study by Travers, who used a NIOX analyzer, and the submitted manuscript may not be directly comparable.

The NIOX analyzer uses chemoluminescence, just as our analyzer as mentioned page 4, paragraph “Methods”, sub-title “FENO”. The NIOX analyzer was used as well by Olin (Olin et al, Chest 2006). In both cited studies and in our study, the guidelines of ATS were used.

As recommended we replaced the reference equations by those of Olin. The methods and results sections were changed accordingly.

3. Atopy has not been assessed objectively in the present study, and I’m not convinced that self-reported allergy is a good alternative for this type of study.

Unfortunately atopy was not determined using Skin Prick Tests. However we considered personal history of allergic markers to be an indirect marker of atopy which was the only marker of atopy available in our study. This marker has also been used in another recent study including FENO measurements: Linn WS, Rappaport EB, Berhane KT, et al. Exhaled nitric oxide in a population-based study of southern California schoolchildren. Respir Res 2009, 10:28.
Moreover, as explained in the «Discussion» paragraph, personal history of allergy has been reported to be as efficient as skin prick tests to common allergens to detect associations with allergic respiratory diseases in some working populations: De Zotti R, Bovenzi M: Prospective study of work related respiratory symptoms in trainee bakers. Occup Envir Med 2000, 57:58-61.

3. Self-reported allergy should not be referred to as “atopy” (page 7, line 1).

We have clarified this point by explaining that we used self-reported allergy in case of physician diagnosis as a marker of atopy in “Methods” paragraph, sub-title “FENO”.

3. Atopy has been shown to be a confounder or effect modifier in other FENO or AHR studies. Given the unexpectedly high prevalence of AHR among the study subjects, I would not be surprised if atopy played an important role.

Indeed, the prevalence of AHR in our study is high, but our population was chosen with an occupation with high AHR. The prevalence of AHR in the study by Massin et al. was 30% in women, not much lower than in the present population.

As mentioned by Dr Smit, atopy could contribute to some extent to the relation between FENO and AHR. It is however highly unlikely that this high prevalence of AHR could be due to atopy in two independent populations sharing the same occupation. An occupational origin is much more likely. This consideration has been added to the discussion.

4. The reference model by Travers includes atopy. It should be clarified how this model has been adapted, as atopy was not available.
As mentioned above, we followed the suggestion of the reviewer and used Olin's reference equations instead.

4. More information should be given on the reference values, i.e. which variables are included in the model.

We used the same variables as Olin, that is height, weight, age, gender, tobacco status, atopy, physician-diagnosed asthma. No subject had asthma symptoms during the previous month or used inhaled steroids, as detailed now in the “Methods” paragraph, sub-title “FENO”.

5. Lifeguards with current asthma were included. How many subjects had asthma? How was asthma defined?

There were two subjects with asthma in childhood only and two subjects with adult incident asthma. None of these subjects was in crisis or under steroid treatment at the time of the study. Asthma was defined by a positive answer to both questions: “did you ever had asthma?” And “was it confirmed by a physician?”

This has been detailed in “Methods” paragraph, sub-title “Symptoms and smoking habits”.

5. In the discussion, it is stated that “it is not in asthmatics”, which seems to contradict the earlier statement.

This occupational population has not been selected based on its asthmatic status but on its occupation.
Both asthmatics in adulthood had AHR and one with asthma in childhood only had AHR. This is now detailed in the “Results” paragraph.

6. The prevalence of AHR was very high, and much higher than in an earlier sample of lifeguards. The potential of selection bias appears to be low, but another explanation of this high prevalence is not given.

We compared the different factors which could have explained this difference and found none. There was no difference in age, sex, tobacco status in lifeguards between the two studies. Exposure in swimming pools was two years longer in the recent study than in the previous one. The mean (SD) NCl₃ concentration was 0.22 (0.10) mg/m³, which is not higher than the 0.24 (0.17) mg/m³ found in the previous study. Only acute work-related nose symptoms were more frequent in the present study (51% versus 31%, p=0.009) but neither ocular nor laryngeal symptoms were statistically different between both studies.

But, as already mentioned, the difference in AHR prevalence is not that important especially not in women.

Discretionary revisions

1. AHR is a hallmark of asthma, but I think it is usually not regarded as a marker of airway inflammation (page 3, line 4).

We have moderated this statement as it is a point of controversy in literature (“widely considered” replaced by “can be considered”).
2. Page 3, 3rd paragraph: “...has a good reproducibility and seasonal variability”. Now it seems as if seasonal variability is an advantage of FENO testing. Could this be clarified?

We meant that factors of FENO variability are known and that a standardisation of measures is possible. We suppressed the detail about season variability that is confusing and adds little to the purpose.

3. It would be interesting to discuss relevant occupational exposures in lifeguards, and their possible relation to AHR or FENO.

This has been added in the “Discussion” paragraph.

Reviewer: Vicky C More

Major compulsory revisions

1. Methods paragraph 1: how was asthma diagnosed? – Physician diagnosis, from the questionnaire or other?

Asthma was defined by a positive answer to both questions: “did you ever have asthma?” And “was it confirmed by a physician?”

This has been detailed in “Methods” paragraph, sub-title “Symptoms and smoking habits”.

2. Methods FENO paragraph: what were the predicteds used?
We used Olin prediction equations as suggested by Dr Smit, see point 4.

2. Travers discusses a mean level for asthmatics of 25.0 ppb and for normals of 17.9 ppb although the percent predicteds shown in this paper are not easily found to relate to these values.

The values in ppb and percent predicted according to Olin as suggested by Dr Smit according to AHR are given in Table 2a.

3. Results: the number of asthmatics included in the study is not stated although in the methods it is written that asthmatics not in crisis without corticosteroids were included. This is important as in the discussion there is a comment about your population not being asthmatic when discussing the AHR.

See answer to Dr Smit point 5.

4. Results in general: actual p values should be shown.

Testing baseline characteristics is not relevant so no p value was included in Table 1.

Table 2a is a descriptive table. However, we added a multiple analysis of normalized dose response slope as a function of log FENO, FEV1 (difference between observed and predicted values), sex, smoking and atopy with p values to supplement the descriptive data in Table 2b.
5. Atopy, smoking and FEV1 should be adjusted for to see if FENO and AHR are still related rather than the other way round.

We apologize if it is not clear but this is exactly what we did. It is stated in the “Results” paragraph page ...: ” No variable – including atopy, smoking, and FEV1 – had an effect on AHR after adjusting for FENO in the logistic regression model.” We changed for: “FENO had an effect on AHR adjusting or not on atopy, smoking, and FEV1 in the logistic regression model. None of these latter factors had a significant effect on AHR when adjusting on FENO.”

5. It is useful to see if these confounders have an effect on AHR too.

We added a sentence stating that these confounders but atopy didn't have a significant effect on NDRS which is a quantitative marker for AHR at the end of the paragraph “Results”.

6. Discussion: The low specificity of using a 70% predicted cut off should be discussed and the relevance of the sensitivity and specificity using a 70% predicted cut off.

Our aim is not to replace methacholine bronchial challenge (MBC) test with FENO measurements, so we don’t advise to take this predicted cut off. It is a way of illustrating the fact that MBC test results are associated with FENO measures.
7. Conclusion: The statement that “using a less than optimal cut off point for abnormal FENO we showed that high FENO values are associated with AHR while low FENO values tended to be associated with normal airway hyperresponsiveness” is not strictly true using the 70% predicted cut off. The latter is correct, but ≥70% predicted FENO picked out almost as many MBC-subjects as MBC+.

We agree with Dr More. We chose arbitrarily a cut off which favours sensitivity (80%) rather than specificity (42% with Olin’s equations). It means that FENO and MBC+ are associated and this association is weak.

Minor essential revisions:

1. Methods, pulmonary function and airway hyperresponsiveness paragraph: an “m” is missing from 3µm.

This has been corrected in this paragraph.

2. Was it 2-min tidal breathing method used for the methacholine challenge? The concentrations are slightly unusual. Has this technique been written up separately? The ATS guidelines paper uses different methacholine concentrations.
It is not a 2-min tidal breathing method. It is an abbreviated version of the MBC test described in Cropp GJA, Bernstein IL, Boushey HA Jr, et al. Guidelines for bronchial inhalation challenges with pharmacologic and antigenic agents. ATS News, Spring 1980; 11–19. We use a single concentration of methacholine and several inhalations delivered by a nebulizer De Vilbiss to reach the desired doses. It has been used in several studies, and in our previous study in lifeguards: Massin N, Bohadana AB, Wild P, Héry M, Toamain JP, Hubert G: Respiratory symptoms and bronchial responsiveness in lifeguards exposed to nitrogen trichloride in indoor swimming pools. Occup Environ Med 1998, 55:258-263.

Discretionary revisions:

1. Figure 1: the data look very spread and the relationship minimal. What is it like as absolute FENO values?

The absolute values are even more spread out. Nevertheless we decided to add a Figure 1b with FENO values in ppb.

2. Results Figures 1 and 2: although the percent predicted information is interesting, because there is such wide ranges in the Travers reference values, figures 1 and 2 would be better shown as absolute values.
The interest of using the equations of Olin is to standardise values by taking into account height, weight, gender, tobacco consumption, physician diagnosed asthma and atopy. As absolute values, the ranges of values are quite wider. Nevertheless we added a Figure 2b with FENO values in ppb.